EFFICACY OF FECAL MICROBIOTA TRANSPLANTATION IN MANAGEMENT OF PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS

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By

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Dedicated

to

my Family

&

Teachers

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Dr. AshokKumar

ABBREVIATIONS

ABIC	Age-Bilirubin-INR-Creatinine Score
AFB	Acid Fast Bacilli
AH	Alcohol-associated hepatitis
AHHS	Alcoholic Hepatitis Histologic Score
ALD	Alcohol- associated liver disease
ALDH	Aldehyde Dehydrogenase
ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
ARE	Antioxidant Response Element
AST	Aspartate aminotransferase
ATP	Adenosine Triphosphate
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
BW	Body Weight
CI	Confidence Interval
CRP	C Reactive Protein
CS	Corticosteroids
CTP	Child - Turcotte - Pugh
DAMP	Damage Associated Molecular Patterns
DFI	Discriminant Function Index
DNA	Deoxyribo Nucleic Acid
ER	Endoplasmic Reticulum

GAHS	Glasgow Alcoholic Hepatitis Score
Hb	Hemoglobin
HDAC	Histone deacetylases
HMGB	High Mobility Group Box
ESPEN	European Society for Clinical Nutrition and Metabolism
FMT	Fecal microbiota transplantation
GCSF	Granulocyte-Colony Stimulating Factor
HCV	Hepatitis C Virus
IL	Interleukin
IU	International Unit
INR	International Normalized Ratio
LPS	Lipopolysaccharide
MDF	Maddrey discriminant function
MELD	Model for End-Stage Liver Disease
mL	Millilitre
NAC	N-acetylcysteine
NAD	Nicotinamide Adenine Dinucleotide
NIAAA	National Institute on Alcohol Abuse and Alcoholism
PAMP	Pathogen associated molecular patterns
pg	Picogram
PT	Prothrombin Time
PTX	Pentoxifylline
RNA	Ribo Nucleic Acid

SAE	Serious Adverse Event
SAH	Severe alcohol-associated hepatitis
SAM	S-Adenosyl methionine
SD	Standard Deviation
SIRS	Systemic Inflammatory Response Syndrome
SOC	Standard of Care
TLC	Total Leucocyte Count
TLR	Toll like receptor
TNF	Tumour Necrosis Factor
UTI	Urinary Tract Infection
WBC	White Blood Cells

ABSTRACT

Introduction: Severe alcoholic hepatitis (SAH) is the most aggressive form of alcohol related liver disease. Corticosteroids remain the only available therapy, with a short term response seen in about 60% of patients but not associated with long term survival benefit leaving the liver transplant to be only curative option for these patients. As alcohol induced intestinal dysbiosis is central to the development of the severe alcoholic hepatitis, there is intense effort in recent past to explore the therapeutic potential of modulating gut microbiota.

Aim: To study efficacy of fecal microbiota transplantation (FMT) in management of patients with severe alcoholic hepatitis.

Materials & Methods: The present study was conducted in Dayanand Medical College and Hospital, Ludhiana in which 20 patients of SAH were evaluated in the study, out of which 10 patients were provided standard of care (SOC) and remaining 10 were imparted FMT along with SOC. The outcome was analyzed at 1, 3 and 6 months.

Results: Mean age was 41±6.91 years. Significant difference in the duration of jaundice between both groups seen; 21.2±9.89 years in FMT and 12.8±4.78 years in SOC group. Both the groups had identical laboratory parameters and prognostic scores (CTP, mDF, MELD, MELD-Na, GAHS) at baseline except mean serum protein, which was significantly more in SOC (6.58±0.57 gm/dL) than FMT group (5.94±0.55 gm/dL). Mean mDF was 71.95±30.88 and 72.48±27.03 in SOC and FMT groups respectively at baseline.11(55%) of 20 patients were steroid-responsive (Lille score ≤0.45); 6 belonged to SOC and 5 to FMT group.FMT group had significant high TLC count and urea level at 1and 3 months.GAHS score was significantly higher in FMT group, 7.5±1.2 in SOC and 8.67±0.5 in FMT group at 1 month; 6 in SOC and 6.67±0.58 in FMT patients at 3 months. Mortality was 20% at 1 month in both groups, 70% mortality recorded in FMT group and 50% in SOC group at 3 months. Mortality was significantly different at end of 6 months, 1(10%) survived in FMT group in contrast to 3(30%) in SOC.

Conclusion: Combination of FMT and steroids has not shown any survival benefits in SAH at 1st, 3rd and 6 months when compared to steroids alone. A significant higher leucocyte count at 1st and 3rd month in FMT group that might have led to significant higher mortality at 6 months.

Keywords:severe alcoholic hepatitis; fecal microbiota transplantation;corticosteroids

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KEY TO MASTER CHART

ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CTP	Child Turcotte Pugh
DM	Diabetes Mellitus
GAHS	Glasgow Alcoholic Hepatitis Score
Hb	Hemoglobin
HE	Hepatic Encephalopathy
HM	Hepatomegaly
INR	International Normalised Ratio
mDF	Maddrey discriminant function
MELD	Model for End-Stage Liver Disease
MN	Management
PH	Past History
PT	Prothrombin Time
TLC	Total Leucocyte Count

INTRODUCTION

Harmful consumption of alcohol leads to approximately 3.3 million deaths every year, corresponding to 5.9% of all deaths globally, out of which, around 50% are caused by alcohol- associated liver disease (ALD).[1] ALD includes a broad spectrum of entities from mild fatty infiltration to steatohepatitis, steatohepatitis with fibrosis, cirrhosis to hepatocellular carcinoma, as a complication of cirrhosis. Progression through these various stages is dependent on continued heavy alcohol use and other risk factors, including female sex, genetic susceptibility, diet and comorbid liver disease.

Alcohol-associated hepatitis (AH) is an important clinical entity as patients with severe alcohol-associated hepatitis (SAH) have extremely high short-term mortality rates and they can develop portal hypertension in the absence of cirrhosis. Furthermore, AH is a well-documented precursor of cirrhosis, with an elevated long-term mortality rate 9 times higher than that for patients with fatty liver alone.[2] The true prevalence of AH is not known, but studies evaluating the histology of patients with ALD suggest that AH might be present in about 10%–35% of alcoholic patients.[3] SAH is the most aggressive form of the disease with a 30-day mortality rate of 20-50%[4] and 3 month mortality of ≥30-70%.[5] The major causes of death are liver failure, sepsis, hepatorenal syndrome and gastrointestinal bleeding.

AH is characterized clinically by rapid onset of jaundice and liver failure. Clinical features associated with severe disease include hepatic encephalopathy, marked prolongation of the prothrombin time, elevation of the serum bilirubin level above 25 mg/dL and development of renal insufficiency. SAH laboratory criteria are defined as modified Maddrey discriminant function (mDF) ≥32 or Model for End-Stage Liver Disease (MELD) ≥21 and/or hepatic encephalopathy.[6] It is characterized by dysregulated and uncontrolled systemic inflammatory response followed by weak compensatory anti-inflammatory response that leads to increased susceptibility to infection and multiple organ failure. Systemic inflammation and poor regeneration are hallmarks of SAH.

The management of SAH patients currently relies on alcoholic abstinence, providing adequate nutrition, corticosteroids and pentoxifylline. Steroids have been used traditionally as the cornerstone of the treatment therapy for SAH since its first demonstration of survival benefit in these patients in late 1980s.[7] However, corticosteroids use in SAH in recent studies have shown that these agents improve survival in these patients only in short term i.e. at 28 days but not associated with any mortality benefit in long term at 90 days or 1 year.[8] In addition, steroids use is associated with significant increased risk of infections. Vergis et al. found that among SAH patients, use of prednisolone was associated with high risk of infections that resulted in high 90-day mortality.[9] Also, there are substantial number of patients who do not respond to steroids and early identification of these patients is important for implementing alternative treatment modalities. Furthermore, a number of patients are not candidate of steroid therapy due to obvious contraindication. Pentoxifylline has been used traditionally in steroids ineligible group but current studies have failed to show any survival benefit with this agent in SAH.[8][10] A number of other therapeutic agents have been studied in patients with alcohol- associated hepatitis. These include androgenic steroids, propyl thiouracil, antioxidants, and specific anti-TNF therapy. None has been shown to improve survival.

Liver transplantation is considered the last option in the wake of medical treatment failure or its contraindication, which in resource and organ scarce country like India does not seem feasible easily. Despite extensive research and clinical trials, the mortality associated with SAH could not be decreased, that indicates to many important facets of this disease, which are yet to be resolved. So, given the high mortality associated with SAH and lack of available treatment options, there is urgent need for new therapeutic modality which may improve outcome in these patients.

ALD is associated with significant change in gut microbial composition and it is now generally accepted that the intestinal flora and gut derived toxins play a critical role in the disease pathogenesis.[11-14] Alcoholics and patient with alcoholic hepatitis display higher levels of

bacterial products in their blood than the healthy humans. Since gut dysbiosis, leaky gut, and products of the gut microbiome reaching the liver that result in to subsequent inflammatory cascade is the major pathological pathway in the development of AH, targeting qualitative and quantitative change of these gut microbiome can prove an important strategy in developing new therapies. The modulation of gut microbiota by fecal microbiota transplantation (FMT) has recently been conceptualized and evaluated as a potential therapeutic strategy in both preclinical and clinical studies. Recent studies which have shown the survival benefit of FMT at 1 year in steroids ineligible SAH have raised the hope that FMT might be a valuable future tool in these patients.[15]

With this background, the present study was conducted to assess whether, the inclusion of FMT with current Standard of Care in SAH patients, leads to any survival benefit and/ or improvement in various prognostic scores.

AIMS AND OBJECTIVES

- To assess the efficacy of Fecal Microbiota Transplantation (FMT) in management of patients with severe alcoholic hepatitis.

REVIEW OF LITERATURE

Amongst heavy drinkers, progression to more severe forms of ALD are not universal, with only 20% developing cirrhosis and up to one-third developing AH. SAH, the most florid form of ALD, has a very high short-term mortality and no specific therapies are available other than steroids.

DIAGNOSIS OF ALCOHOLIC- ASSOCIATED HEPATITIS (AH)

AH, per se, is a clinical syndrome with a distinct histopathological correlate, called alcoholic steatohepatitis. The histological features of AH may be present in patients with no symptoms and mild laboratory abnormalities. Its histological features consist of neutrophilic lobular inflammation, degenerative changes in hepatocytes (ballooning and Mallory- Denk bodies), steatosis, and pericellular fibrosis.[16] The National Institute on Alcohol Abuse and Alcoholism (NIAAA), Alcoholic Hepatitis Consortia defines AH to include the following:- [17]

- The onset of jaundice within 60 days of heavy alcohol consumption (more than 40 g/day for females and 60g/day for males) for a minimum of ≥6 months;
- with serum bilirubin more than 3 mg/dL;
- elevated aspartate aminotransferase (AST) to 50 U/L to 400 U/L;
- AST: ALT (alanine aminotransferase) ratio of more than 1.5;
- No other cause of acute hepatitis.

Based on expert recommendation, AH can be further characterized as:

a) **Definite AH**: in which both clinical criteria and confirmation of diagnosis on liver biopsy are met;

b) **Probable AH**: defined in a patient who has both alcohol use disorder and hepatitis, and investigations have ruled out other causes of liver disease but a biopsy confirmation is lacking;

c) **Possible AH**: defined in patients in whom the clinical diagnosis of AH is suspicious, in the presence of other clinical events such as ischemic hepatitis, drug induced liver injury; associated viral infections; or in the presence of atypical AST and ALT pattern.

In this context to identify patients with more severe disease, a variety of prognostic scores have been developed with the aim to select patients at high risk of early death. There are various disease-specific prognostic models (mDF: Maddrey's Discriminant Function; GAHS: Glasgow Alcoholic Hepatitis Score; ABIC: Age-Bilirubin-INR-Creatinine Score; Lille model; MAGIC: Model for Alcoholic hepatitis to Grade the severity in an Asian patient Cohort,) and a non-disease specific model (MELD: Model for End-Stage Liver Disease).[18-23]

Table 1: Components of clinical scoring systems to assess prognosis in AH

	Bilirubin	PT/ INR	Cr/ BUN	WBC	Age	Albumin	K^+	Change in bilirubin Day 0- 7
mDF[18]	+	+	-	-	-	-	-	-
MELD[19]	+	+	+	-	-	-	-	-
GAHS[20]	+	+	+	+	+	-	-	-
ABIC[21]	+	+	+	-	+	+	-	-
Lille[22]	+	+	+	-	+	+	-	+
MAGIC[23]	+	+	+	-	-	-	+	+

Maddrey's Discriminant Function (mDF)

The original description of Discriminant Function Index (DFI) was by Maddrey and colleagues in a placebo- controlled study to see the benefit of corticosteroids in patients with AH.[18] This score was modified later and called Modified Discriminant Function (mDF). Patients with mDF ≥ 32 are consider to have SAH and they have high risk of short-term mortality (30%-50% at 28 days). In contrast, a MDF <32 identifies those with mild/moderate AH, conferring low, but not zero, risk of mortality with supportive care.

MELD score (Model for End-Stage Liver Disease)

MELD score is already a well-validated prognostic score in cirrhosis. Its usefulness in assessing the short-term prognosis of AH has been studied in retrospective studies, which suggest that patients with an MELD score above 20 are defined as SAH and are at a high risk of 90-day mortality.[24] Monitoring of MELD score at regular intervals with a change of 2 or more points over the first week on treatment has independently predicted mortality.[19]

GAHS (Glasgow Alcoholic Hepatitis Score)

GAHS is calculated considering age, peripheral total leucocyte count, serum bilirubin (at days 1 and 6-9), blood urea nitrogen and PT. GAHS score > 9 points characterize severe AH. The one-month survival in patients with GAHS <9 was 87% compared to 46% in those with score ≥9. The limitation is lack of international validation.[20]

Age - Bilirubin - INR - Creatinine (ABIC) score

A new scoring system called the Age - Bilirubin - INR - Creatinine (ABIC) score based on age, serum bilirubin, serum creatinine and INR during admission with a cut-off value of 6.71 to 9, identified patients with low (0%), intermediate (30%), and high risk (75%) of death at three months. The ABIC score was also useful in determining 1-year mortality.[21]

Lille score

If the Lille score is >0.45 at day 7: it depicts non responder to corticosteroids. Survival is 25% as compared to 85% at 6 months, if the Lille score is > 0.45.(22) Lille response can also be divided into complete responders (score ≤0.16), partial responders (0.16–0.56), and null responders (≥0.56) with one-month survival rates of 91%, 79% and 53%, respectively.[25]

MAGIC score

In Asian patients hospitalized for AH, a new model has been found called MAGIC to predict liver-related death.[23] It mainly highlights on the

forecast the natural outcomes of untreated patients with AH, prognostic value of hyperkalemia in AH.

Child - Turcotte - Pugh (CTP) score

It is not traditionally employed for predicting mortality of SAH. However, it helps to tell mortality at 3 and 6 months.[26]

Alcoholic Hepatitis Histologic Score (AHHS)

Another score, an alcoholic hepatitis histologic score (AHHS) has been proposed recently to predict survival outcomes correctly in patients with biopsy-proven, AH.[27] The calculation of AHHS is made by grading the extent of fibrosis, the degree of neutrophilic infiltration, bilirubinostasis patterns, and megamitochondria.[28]

Other factors independently associated with higher mortality from AH include older age, alcohol consumption >120 g/d, upper gastrointestinal bleed, bilirubin to gamma glutamyl transferase ratio >1, interleukin (IL) −6 levels ≥38.66 pg/mL and in-vitro measure of steroid sensitivity.[29][30]

Despite all these scoring systems, in the clinical practice, mDF > 32 or MELD > 20 are used to guide initiation of treatment with corticosteroids. A Lille score > 0.45, at day 7, triggers discontinuation from CS.[31][32] Patients with AH with hepatic encephalopathy are also considered to have SAH irrespective of the mDF score.

SAH is characterized by dysregulated and uncontrolled systemic inflammatory response followed by weak compensatory anti-inflammatory response that leads to increased susceptibility to infection and multiple organ failure. Owing to such a high mortality and limited available treatment modalities, mandates to understand the disease pathogenesis meticulously so that future therapies can be developed.

PATHOGENESIS OF ALD

1. Ethanol metabolism and toxic metabolites:

In hepatocytes, the primary pathway of ethanol metabolism is through acetaldehyde. Acetaldehyde reacts with proteins (e.g. –cysteines) and forms DNA adducts which causes cellular toxicity.[33] It has also been shown to impair mitochondrial glutathione transport and to sensitize hepatocytes to TNF-α–mediated killing.[34]

The oxidation of ethanol uses nicotinamide-adenine dinucleotide (NAD+) and thereby causes a shift in the ratio of reduced NAD (NADH) to NAD+ to a more reduced state.[35] This change in the redox state can impair normal carbohydrate and lipid metabolism; multiple effects ensue, including a decrease in the supply of ATP to the cell and an increase in hepatic steatosis.

2. Other Metabolic Mechanisms

2a. Oxidative Stress

Indicators of oxidative stress are several indirect markers: e.g., protein thiol or carbonyl products, isoprostanes, malondialdehyde**,** oxodeoxyguanosine. The stimulus for oxidative stress in the liver comes from multiple sources. The CYP2E1 system induced by chronic alcohol intake leaks electrons to initiate oxidative stress.[36] Nonparenchymal cells and infiltrating inflammatory cells are another major source of pro-oxidants.

2b. Mitochondrial Dysfunction

Structural and functional mitochondrial abnormalities are well documented in ALD and contributes to oxidative stress.[37] The increased NADH/NAD+ ratio caused by ethanol intake favours superoxide generation, thereby increasing flow of electrons along respiratory chain. In ALD, the transport of glutathione into mitochondria is impaired, and selective mitochondrial glutathione depletion is observed. This process is likely a cause of global alterations in mitochondrial function related to alcohol abuse and ALD.

2c. Abnormal Metabolism of Methionine, S-Adenosyl methionine & Folate

Activity of enzyme methionine adenosyl is depressed in ALD.[38][39] Exogenous administration of SAMe corrects the deficiency and attenuates the severity of many experimental forms of liver injury. SAMe has been shown to maintain mitochondrial glutathione levels. SAMe, but not other glutathione prodrugs, prevents mitochondrial glutathione depletion in experimental ALD (possibly by protecting mitochondrial glutathione transport systems).[40] Keap1-Nrf2 (NF-E2-related factor 2) -ARE (antioxidant response element) pathway plays a key role in cellular responses to stress. In experimental cholestatic liver disease, Nrf2 binding decreases, and this is partially prevented with SAMe therapy. Therefore, SAMe therapy may help maintain GSH levels as well as induce other antioxidant pathways through maintenance of appropriate Nrf2 binding.[41][42] SAMe also decreases lipopolysaccharide (LPS)-stimulated TNF release and increases interleukin (IL)-10 release in a monocyte cell line.[39] Although serum SAMe levels are decreased in patients with ALD, levels of the downstream products S-adenosylhomocysteine (SAH) and homocysteine are elevated.[38][43][44] Elevated SAH levels have been shown to sensitize hepatocytes to TNF-mediated destruction, and SAH may be a critical physiologic sensitizer of TNF-mediated killing in liver injury.[44]

2d. Hypoxia

Chronic alcohol intake increases oxygen uptake by the liver and increases the lobular oxygen gradient. Hepatocyte-specific HIF-1α which is regulated by microRNA-122vhas been shown to be up-regulated in alcohol-fed mice and to play a role in hepatic lipid accumulation.[45] There is increase in HIF-1α in ALD is, a negative regulator of HIF-1α. Levels of microRNA-122 are significantly decreased in the liver in ALD in mice and in humans. The low miR-122 levels in ALD are due to a direct inhibitory effect of alcohol on the transcription of this microRNA.[46] on contrary, low intestinal HIF-1α in ALD contributes to increased intestinal permeability. Indeed, one mechanism of the beneficial action of the

probiotic Lactobacillus GG in experimental ALD appears to be maintenance of intestinal HIF.[47][48]

2e. Endoplasmic Reticulum Stress, Impaired Proteasome Function, and Autophagy

The endoplasmic reticulum (ER) stress which is dealt by unfolded protein response can be detrimental (usually in the long term). Some potential inducers of ER stress in ALD include elevated homocysteine levels, acetaldehyde and acrolein adducts, and oxidative stress.[49][50] ER stress leads to activation of the ER-associated molecule, STING, that triggers phosphorylation of the interferon regulatory factor 3 which interacts with mitochondrial apoptotic proteins to result in hepatocyte damage, apoptosis, and the release of damage-associated molecular patterns (DAMPs), such as ATP and uric acid, that then contribute to inflammasome activation.[51]

Both of the major pathways that degrade most cellular proteins in eukaryotic cells, the ubiquitin-proteasome system and autophagy, are affected in ALD.[52] Animal studies have demonstrated decrease in proteolytic activity of the proteasome can lead to abnormal protein accumulation, including accumulation of oxidized proteins.[53] The decrease in proteasome function correlates significantly with the level of hepatic oxidative stress. Studies in rats have also shown that alcohol consumption inhibits multiple key steps in autophagy.[54]

3. Immune and Inflammatory Mechanisms

3a. Gut-liver axis & Pathogen associated molecular patterns (PAMPs):

The gut-liver axis refers to the bidirectional relationship between the gut and its microbiota, and the liver, established through the portal vein, which carries gut-derived products to the liver, and the feedback of bile and antibodies from the liver to the intestine. The intestinal mucosal and vascular barrier is the functional and anatomical structure that serves as a playground for the interactions between the gut and the liver, limiting the systemic dissemination of microbes and toxins while allowing

nutrients to access the circulation and to reach the liver. The control of microbial communities is critical to maintaining homeostasis of the gut-liver axis, and as part of this bidirectional communication the liver shapes intestinal microbial communities.

Indeed, in the 1960s, it was shown that germ-free rodents or rodents treated with antibiotics to “sterilize the gut” were resistant to nutritional and toxin-induced liver injury. Early studies showed that rats fed a choline-deficient diet developed cirrhosis, which could be prevented by oral neomycin.[55] Subsequently, antibiotics, prebiotics, and probiotics have all been used to prevent experimental alcohol-induced liver injury.[56] Numerous clinical studies also have demonstrated that plasma endotoxin levels are significantly elevated in patients with different stages of ALD and causes hepatic injury. Lipopolysaccharide (LPS) is a prototype PAMP that represents a strong inflammatory signal for the host through recognition by Toll-like receptor 4(TLR4).[57] With increased gut leakiness, many different PAMPs can translocate to the liver via the portal circulation and contribute to inflammatory cell activation.[58]

Alcohol- induced gut barrier dysfunction and endotoxemia are multifactorial events, with altered microflora and impaired intestinal integrity among the causal factors. Alcohol promotes the overgrowth of Gram negative bacteria, studies on gut flora from alcoholics in an inpatient treatment program have demonstrated altered microflora composition, with decreased numbers of Bifidobacteria spp. and Lactobacilli spp.[59] One human study found depletion of Akkermansia muciniphila in humans with ALD and showed that replacement of Akkermansia spp. can ameliorate alcohol-induced liver disease in mice.[60] Another study found that after a 10-day alcohol feeding binge, a decrease in intestinal A. muciniphila was an early change in the gut microbiota related to alcohol.[61] Multiple animal studies have documented altered gut flora with chronic alcohol feeding. [62]

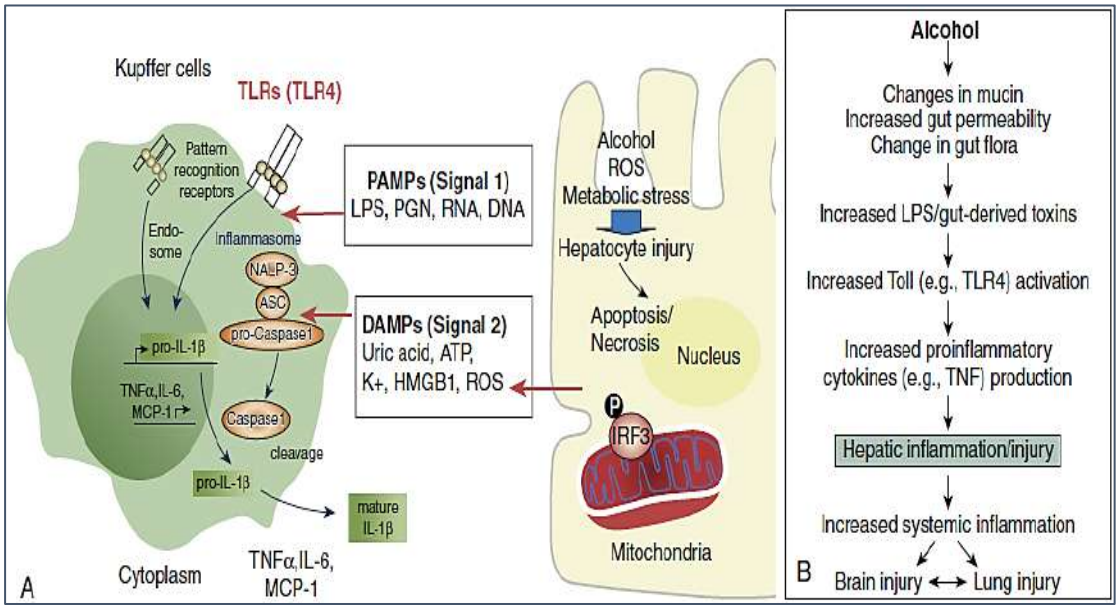

Figure 1: Gut- Liver axis in pathogenesis of ALD

Alcohol and its metabolite, acetaldehyde, induce intestinal permeability to various macromolecules, including LPS, in both human subjects and animal models of ALD.[43] Indeed, decreased tight junction (ZO-1) protein levels were observed in sigmoid colonic biopsies of human alcoholics compared with healthy controls. The decrease was attributed to an increase in miRNA-212 expression observed in alcoholic subjects compared with controls.[63] Increased intestinal production of proinflammatory cytokines, such as TNF and IL-6, can also contribute to alcohol-associated endotoxemia by altering tight-junction morphology and distribution, thereby creating a self-perpetuating vicious cycle that can amplify bacterial translocation.[64] Finally, emerging evidence suggests that intestinal fungi also contribute to ALD. Alcohol-dependent patients have been shown to display reduced intestinal fungal diversity and overgrowth of Candida spp.[65] Consistent with this finding, antifungal therapy reduced the alcohol-related intestinal fungal overgrowth and increased serum β-glucan levels.[65]

3b. Inflammasome activation and Damage -associated Molecular Patterns

Inflammasomes stimulation mediates the caspase-1 activation that results in proteolytic cleavage of pro-IL-1β and IL-18 into bioactive forms.[66] Inflammasome activation requires 2 signals; the first, usually a TLR-mediated signal that induces pro-IL-1β production and the second, usually a DAMP-induced signal.[67] The levels of DAMPs, including uric acid and ATP, are increased in ALD, and inhibition of these sterile danger signals can prevent inflammasome activation in mice.[68] Inhibition of the actions of IL-1β with a recombinant IL-1 receptor antagonist has been shown to improve ALD in mice and accelerate liver regeneration after chronic alcohol exposure.[69] Recently other DAMPs, for example, high mobility group box-1 (HMGB-1), IL-33 and its decoy receptor soluble ST2 (IL-1 receptor like 1)—have been shown to play different roles in early and late stages of ALD in mice. [70][71]

3c. Dysregulated Cytokine Production

In ALD, monocytes and Kupffer cells are primed to increase production of TNF, and hepatocytes are sensitized to TNF killing. Serum concentrations of TNF-inducible cytokines and chemokines, such as IL-6, IL-8, IL-18, monocyte chemoattractant protein 1, and others, are elevated in patients with AH or cirrhosis, and the levels often correlate with markers of an acute-phase response, reduced liver function, and poor clinical outcomes.[56] In contrast, monocyte and Kupffer cell production of protective anti-inflammatory cytokines, such as IL-10, is decreased.[72]

3d. Immune Responses to Altered Hepatocellular Proteins

AH may persist histologically for many months after exposure to ethanol has ceased, suggesting an ongoing immune or autoimmune response. Certain autoantibodies directed against phospholipids, ADH, heat shock protein, and other potential antigens have been detected in ALD. Some studies also have linked genetic susceptibility and autoimmunity in ALD.[73]

4. Genetic and Epigenetic Factors

Genetic polymorphisms in alcohol-metabolizing systems such as CYP2E1 and ADH likely play a role in susceptibility to ALD. People with nucleotide substitution from glutamine to lysine in aldehyde dehydrogenase (ALDH) have significantly low or absent ALDH activity, leading to acetaldehyde accumulation.[74][75] CYP2E1 is inducible, with the activity increased by 20-fold after alcohol consumption. The C2 variant has higher activity than that of C1, leading to the assumption that it will lead to high levels of acetaldehyde, oxidative stress, and liver injury.[76][77] A sequence variation within the gene coding for patatin like phospholipase encoding 3 (PNPLA3, rs738409) has been shown to be a robust genetic risk factor for progressive ALD.[78]

Epigenetic mechanism such as acetylation of the core histones result from the balance between the opposing activities of histone acetyltransferases and histone deacetylases (HDACs). Binge alcohol exposure significantly alters messenger RNA expression of liver class I, II, and IV HDACs.[79] Another type of epigenetic effect is microRNA. For example, miRNA 155 (miR-155) has been shown to modulate LPS-induced TNF-α production in Kupffer cells and TNF production in macrophages from patients with ALD.[80]

5. Emerging Mechanisms

Endogenous cannabinoids, which mediate their effects through specific cannabinoid receptors, CB1 and CB2, appear to have a role in ALD. Studies have demonstrated that inhibition of CB1 receptors can cause weight loss and attenuate fatty liver and hyperlipidemia in animal models of obesity and steatohepatitis. Moreover, CB1 blockade reduces hepatic fibrosis in a variety of animal models of cirrhosis.[81]

Malnutrition has re-emerged as another mechanism of interest. Alterations in micronutrients, such as vitamins A, D, and zinc, as well as macronutrients, such as dietary fat, are increasingly recognized to play a role in the development and progression of ALD.[82]

Current Standard of Care for Severe Alcoholic Hepatitis

1. General measures

Initial evaluation of patients with AH should include a careful history of alcohol consumption, including recent use, quantity, and frequency of intake. The cornerstone of therapy for all stages of ALD is abstinence from drinking alcoholic beverages. Features of systemic inflammatory response syndrome (SIRS) in patients with SAH indicate a higher probability of organ failure as well as the possibility of infection. Analysis of blood and urine cultures and ascites should be performed regardless of fever. Prophylactic coverage with antibiotics should be considered while awaiting results of cultures. If identified, active infections require treatment prior to initiating therapy for SAH that can impair the immune response. Considering the potential risk of Wernicke's encephalopathy, supplementation with B-complex vitamins should be given. Other general approaches include treatment of hepatic encephalopathy (lactulose, rifaximin) and treatment of ascites (salt restriction). Avoidance of diuretics and nephrotoxic drugs as these patients are at increased risk of developing acute kidney injury.

2. Nutrition

Several studies have highlighted that protein energy malnutrition is present in almost every patient with severe AH, and is associated with poor prognosis.[83] The European Society for Clinical Nutrition and Metabolism (ESPEN) recommend a daily energy intake of 35–40 kcal/kg of body weight (BW) and a daily protein intake of 1.2–1.5 g/kg of BW in patients with SAH. However, these objectives are often difficult to achieve in clinical practice. Therefore, the use of tube feeding is strongly recommended if patients are not able to maintain adequate oral intake.

3. Glucocorticoids

Glucocorticoids were first used to treat AH almost 50 years ago, primarily because of the inflammatory features of the illness.[84] Since then, more than 20 randomized, controlled trials of glucocorticoids have

been conducted in patients with SAH as defined by an mDF greater than 32. More recently, a meta-analysis using primary data from 11 trials concluded that glucocorticoids reduce 28-day mortality of severe AH but did not affect 6-month mortality.[85] A 2019 systematic review of 16 trials using the Cochrane methodology concluded that there was no benefit from glucocorticoids and that 15 of the 16 trials had a high probability of bias.[86] The STOPAH trial randomized 1103 patients with severe AH in Europe between 2011 and 2014 to receive prednisolone 40 mg (equivalent to 32 mg of methylprednisolone) daily, pentoxifylline (PTX) 400 mg 3 times daily, the combination of prednisolone and PTX, or placebo. It concluded Prednisolone was associated with a reduction in 28-day mortality that did not reach significance and with no improvement in outcomes at 90 days or 1 year. Serious infections occurred in 13% of the patients treated with prednisolone versus 7% of those who did not receive prednisolone (P=0.002).[8] A network meta-analysis using results from 22 recent trials involving 2621 patients with SAH showed that treatment with glucocorticoids alone or in combination with PTX or the antioxidant N-acetylcysteine (NAC) reduced 28-day but not 90-day mortality compared to standard care based on moderate-quality evidence.[87] Based on the current evidence current guidelines of both the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver that recommend glucocorticoid treatment for patients with SAH without active infections.[88][89]

Therapies lacking evidence for efficacy in treatment of Alcoholic Hepatitis

1. **Pentoxifylline (PTX)**

Although PTX was reported to reduce mortality in patients with severe AH (MDF >32),[90][91][92] studies combining PTX with glucocorticoids did not show any benefit in the survival of patients with severe AH.[93][8][5] Two meta-analyses and 1 network meta-analysis concluded that there were no differences in short-term mortality related

to PTX.[87][10][94] Based on these findings, PTX is not recommended for use in patients with severe AH.

2. Anti- Tumor Necrosis Factor α Therapy

Although preliminary evidence suggested that anti-TNFα therapy might be beneficial in SAH[95][96] subsequent larger trials of etanercept (antibody to TNFα receptor) and the combination of anti-TNFα therapy with glucocorticoids led to a higher risk of infections and a higher mortality rate than standard care.[97] These trials have generally led to an abandonment of anti- TNFα therapy in AH.

Novel Therapies for Alcoholic Hepatitis

Understanding the pathogenesis of AH provides the potential to develop new therapies based on the mechanisms of injury.

1. Prevention of the Development of Steatosis

1a. Obeticholic Acid

Obeticholic acid, a bile acid derivative with anticholestatic and hepatoprotective properties, acts via the FXR pathway, is a promising therapeutic target for the treatment of NAFLD.[98][99] Given the similarities between ALD and NAFLD, obeticholic acid could lower the severity of alcoholic steatosis and prevent downstream effects. Studies are currently underway to evaluate the role of obeticholic acid in AH.

1b. 5-Cholesten-3b, 25-Diol 3-Sulfate (25HC3S)

A novel oxysterol synthesized by oxysterol sulfation from 25-hydroxycholesterol, downregulates lipid metabolism and inflammatory responses acting through decreases in LXR and SREBP-1c activity and increases in PPARγ.[100] In an open-label study 17 of 19 patients dosed with up to 150 mg of 25-HC3S had Lille scores of less than 0.45 by day 7 after treatment.[101] The Lille scores in these patients were significantly better than those of historically matched lucocorticoid- treated patients.

1c. Metadoxine

Metadoxine is an ion pair salt of pyridoxine and pyrrolidone carboxylate. There is some evidence that metadoxine increases glutathione levels and reduces steatosis in animals fed alcohol and may act as an antioxidant.[102] One study showed a statistically significantly improved 90-day survival in patients treated with a combination of metadoxine and either PTX or prednisone compared to PTX or prednisone alone.[103]

2. Inflammation Mediated by the Innate Immune Response

2a. Interleukin 1 Receptor Blockers

These agents have recently shown some beneficial role in SAH patients. The combination of anakinra for 14 days plus zinc for 180 days plus PTX for 28 days resulted in similar survival at 28 days and a 22% better survival at 90 and 180 days compared to patients treated with methylprednisolone; however, the difference was not statistically significant.[104]

2b. Caspase Inhibitors

In the sterile inflammation pathway activated by LPS/Toll-like receptor (TLR) 4, caspase-1 ultimately initiates cell death. A phase 2 trial in patients with NAFLD showed promise for selonsertib, an apoptosis signal-regulating kinase inhibitor,[105] but a subsequent trial in patients with severe AH failed to show any significant difference between prednisolone plus selonsertib and prednisolone.[106]

3. Stimulation of Liver Regeneration

3a. Granulocyte-Colony Stimulating Factor

A pilot study of G-CSF plus standard care, including PTX, showed improved survival of 78.3%, compared to 30.4% (p=.001) in patients treated with standard care alone at 90 days.[107] G-CSF was also reported to improve 90-day survival in glucocorticoid non-responders (Lille score >0.45).[108] Interleukin 22 A phase 2 trial of IL-22 (F-652) showed improved Lille scores at 7 days and MELD scores at 28 and 42

days, along with upregulation of markers of regeneration and downregulation of markers of inflammation in 18 patients with AH.[109] No serious adverse events were reported in this population of patients with AH.

3b. Immunoglobulin- Rich Bovine Colostrum

Immunoglobulin- rich bovine colostrum has been shown to have immunomodulatory effects, lower serum endotoxin levels in animal studies, and improve gut permeability in critically ill patients.[110]

4. Modification of the Microbiome

4a. Probiotics

In a small study of patients with mild-moderate AH, short-term supplementation of Bifidobacterium bifidum and Lactobacillus plantarum 8PA3 restored gut flora and improved laboratory markers of liver injury (AST, ALT, GGT, lactate dehydrogenase, serum bilirubin).[59] Lactobacillus rhamnosus GG supplements may improve mucosal integrity, inhibit endotoxin activation of TLR4, reduce TNFα production, and, thus, lower alcohol-mediated inflammation.[111][47]

4b. Fecal Microbiota Transplatation (FMT)

Gut dysbiosis, greater gut permeability, and increased gut microbial products in the portal circulation are the most commonly reported triggers initiating alcoholic hepatitis, prompting the activation of the innate and adaptive immune systems, hence the hepatic insult. Gut microbiome modulation may be an important and promising way of dealing with alcoholic liver disease. One emerging strategy to address dysbiosis therapeutically is with the administration of FMT, i.e. the transplant of a homogenised filtered faecal sample from a healthy donor directly into the gastrointestinal tract of a patient with dysbiosis. The aim of FMT in ALD to disrupt the pathological pathways aforementioned by restoring the gut microbiome.

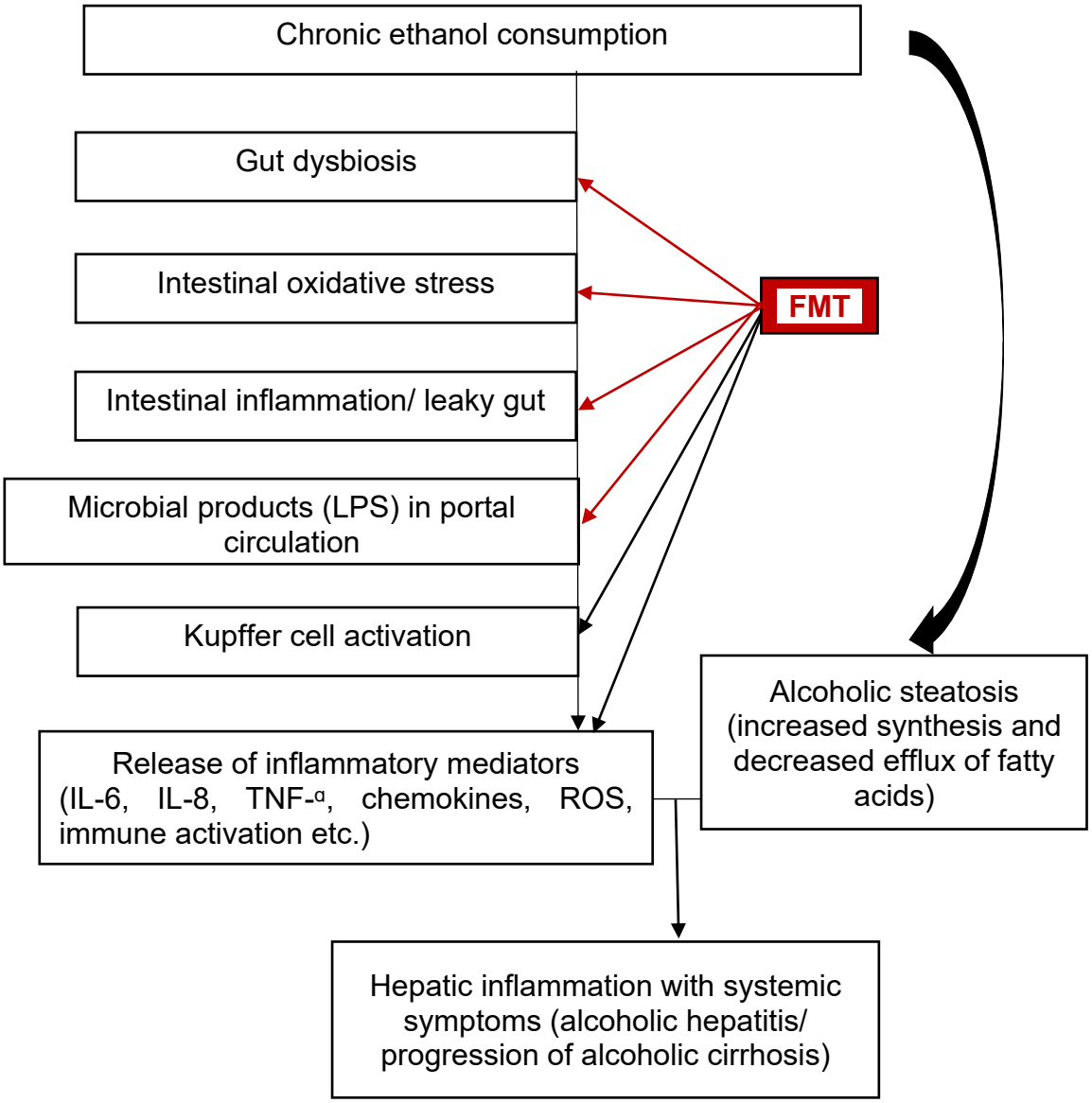
Chronic ethanol consumption
Gut dysbiosis
Intestinal oxidative stress
FMT
Intestinal inflammation/ leaky gut
Microbial products (LPS) in portal circulation
Kupffer cell activation
Alcoholic steatosis (increased synthesis and decreased efflux of fatty acids)
Release of inflammatory mediators (IL-6, IL-8, TNF-α, chemokines, ROS, immune activation etc.)
Hepatic inflammation with systemic symptoms (alcoholic hepatitis/ progression of alcoholic cirrhosis)

Figure 2: Gut liver axis

Procedure of FMT

1. FMT donation and processing

Donor screening is of the utmost importance and should identify a healthy donor without any active infections by conducting screenings for hepatitis B surface antigens, anti-hepatitis C antibodies, human immunodeficiency viruses 1 and 2, and venereal disease research laboratory testing. The donor's stool should also test negative for ovarian cysts, Rotavirus antigens, Helicobacter pylori antigens, Cryptosporidium, Isospora (AFB stain), and C. difficile toxins. Donors

should not have experienced recent alcohol intake (in the last 90 days), altered bowel movements, or recent antibiotic use (in the last 90 days).

The donor should also be free of any significant co-morbidities or chronic ailments and preferably aged between 18 and 60 years. In general, a young, healthy, lean, individual, preferably a relative (who possibly would share the same kitchen/ human leukocyte antigen alleles), is an ideal donor for FMT. A stool sample should be collected in the early morning a clean plastic container, preferably at a place very near to the processing unit (the FMT laboratory) to be processed in an automated system for safe and early disposal of the filtrate containing the microbiome suspension without fibrous residue.

2. Fecal preparation for FMT

Fresh fecal samples are preferred over frozen or stored samples given safety, efficacy, and viability concerns. Some reports suggest the level of efficacy is equal between the stored samples and the stored frozen samples[112] but most prefer fresh samples due to concerns about the loss of a proportion of the viable microbiome.[113][114][115] At many large-volume FMT centers, including ours, the FMT workflow (collection, transportation, preparation, and delivery) is designed to be completed within 3 hours and fresh stool samples are always preferred, although further research in this regard is warranted. Automated GenFMTer purification systems (FMT Medical, Nanjing, China) are also useful in facilitating clean handing of the samples and packaging of the fecal slurry in easily and safely transportable parcels.[116][15][117]

3. FMT route of administration

As of today, the best route of administration is not confirmed from amongst the oral, nasogastric, nasoduodenal, nasojejunal, endoscopic, rectal, and colonoscopic options depending on the disease condition[118][116] and there are no head-to-head comparisons available in the literature. However, in cirrhotics with dysbiosis predominantly involving the proximal small bowel, the upper gastrointestinal route of FMT administration seems more logical.[112]

CURRENT DATA FOR FMT IN ALD

The concept of FMT in ALD is evolving but recent studies has shown that this might prove to be an efficient tool in these patients. In a pilot study of FMT in steroid ineligible patients, Philips et al. demonstrated that 1 week of FMT was effective and safe in patients with SAH and improved indices of liver disease severity and survival at 1 year. Survival was significantly better in the FMT group compared with historical controls (87.5% vs 33.3%). Microbiota analysis showed no difference in phyla composition of donors and recipients at baseline. Firmicutes dominated in donors and recipients at 1 year, Proteobacteria reduced, and Actinobacteria increased post-FMT in recipients. Changes in relative abundance of certain pathogenic species, such as Klebsiella pneumonia and non-pathogenic species, such as Enterococcus, Bifidobacterium longum, and Megasphaera elsdenii were noteworthy in this study. At the end of 1 year, methane metabolism, fluorobenzoate acid degradation pathway (mediated by Pseudomonas and Escherichia coli groups) and bacterial invasion of the epithelial cells were down-regulated post-FMT. Whereas, metabolism which were initially down-regulated at the baseline such as bile secretion, carotenoid biosynthesis, and pantothenate biosynthesis pathways improved to near normal levels following FMT.[15]

In 2018, Philips et al. compared the outcomes in SAH patients on nutritional therapy, corticosteroids, pentoxifylline and healthy donor FMT which showed that the proportions of patients surviving at the end of both 1 and 3 months was maximum in FMT group i.e. 75%. Whereas, it was 63%, 47%, 40% in the steroids, nutrition, pentoxifylline respectively at the end of 1st month and 38%, 29% and 30% respectively at the end of 3rd month. When compared with FMT, relative risk and hazard ratios for mortality were higher in all the other groups. Following FMT, distinct and beneficial modulation of stool microbiota and pathways of dysregulated metabolism, infections, inflammation, and oxidative stress in SAH patients were noted in tandem with improved clinical outcomes.[119]

Regarding use of FMT in cirrhotic patients, an open-label, randomized clinical trial by Bajaj et al. in 20 outpatient males with

cirrhosis with recurrent HE was conducted with 1:1 randomization into standard of care (SOC) and FMT group. A single FMT enema from the same donor was administered to FMT-randomized patients post 5 days of broad-spectrum antibiotic pre-treatment. FMT participants experienced lesser SAEs (20%), as compared to SOC participants (80%) at the end of 5 months follow- up. Further HE episodes were not observed in FMT participants, while, 5 SOC developed HE episodes. Model for End-Stage Liver Disease (MELD) score transiently worsened post antibiotics, but reverted to baseline post-FMT. Cognition improved and diversity with beneficial taxa increased in the FMT group, but not the SOC group.[120]

Long-term safety and efficacy of FMT in cirrhotic patients was evaluated by Bajaj et al. where they followed up participants of both standard of care (SOC) and FMT group for >12 months and up to 15 months and noted that FMT intervention was well tolerated with a non-concerning long-term safety profile, without infections or the need for new antibiotic initiation. There were significantly more hospitalizations and HE episodes in the SOC arm compared with the FMT arm during the long-term follow-up. Moreover, cognitive function, which had improved in the FMT arm at day 20 after FMT, remained significantly better in the FMT arm compared with the SOC arm on long term testing.[121]

In a study to determine the effect of colonization using human donors in germ-free (GF) mice on the gut–liver–brain axis by Liu et al., it was demonstrated that differential colonization of GF mice with stools from healthy controls versus patients with cirrhosis with or without FMT is associated with changes in brain inflammatory expression. The colonization of GF mice with stool obtained from patients with HE after antibiotics results in greater brain inflammation compared with the mice that received faeces obtained after successful FMT. These changes in brain inflammatory expression after FMT were not seen to the same extent using GF supernatants, signifying that transferred microbiota mediated the neuroinflammation.[122]

The current standard of care for SAH has several limitations as the steroids are the only definite available options for treatment but only up to one-third of the patients are eligible for steroid therapy. Additionally, steroids have their own issues: a portion of patients do not respond, while there is doubtful long-term benefit in those who do and a large proportion are ineligible to receive steroids entirely and hence have no definitive options for treatment. As such, there is a large gap between the problem and the available solutions.

FMT is expected to have a role in managing ALD in general and SAH in particular by correcting dysbiosis, the primary insult. Available preliminary data are promising and FMT might become the standard of care in the near future for managing alcohol-related liver diseases, especially SAH, with greater applicability, improved acceptability, and minimal side effects. This study aims to delineate the role of FMT in SAH patients and may help to understand the future prospective of FMT.

MATERIALS AND METHODS

STUDY DESIGN

It was a randomised prospective study conducted on the patients of severe alcoholic hepatitis admitted in Department of Gastroenterology, Dayanand Medical College and Hospital, Ludhiana, India.

SOURCES OF DATA

Patients diagnosed with SAH during study period who were admitted in the Department of Gastroenterology, DMC&H. 24 patients were enrolled for the study, out of which 4 patients lost to follow up and excluded from study. 20 patients were finally analysed.

METHODS OF COLLECTION OF DATA

Diagnosis of alcoholic hepatitis (AH) was made on basis of clinical criteria i.e. onset of jaundice within 60 days of heavy consumption (>50 g/day) of alcohol for a minimum of 6 months, a serum bilirubin level greater than 3 mg/dL, an elevated serum AST level (50 to 400 U/L), a serum AST:ALT ratio greater than 1.5, and no other obvious cause for hepatitis.

Among patients of AH who had modified Maddrey discriminant function (mDF) ≥32 or Model for End-Stage Liver Disease (MELD) ≥21 and/or hepatic encephalopathy were labelled as severe alcoholic hepatits (SAH) and enrolled in study.

INCLUSION CRITERIA

- Patients with diagnosis of SAH and eligible for steroids therapy.
- Patient with resolved sepsis and acute kidney injury were enrolled into the study.
- Men and women age > 18 years and above

EXCLUSION CRITERIA

- Documented cirrhosis prior to onset of illness
- Active sepsis
- Serum creatinine > 1.5 mg/dl
- Acute pancreatitis
- Other concomitant causes of liver disease: viral hepatitis, autoimmune liver disease, metabolic liver disease, vascular liver disease
- Ultrasound evidence of grade 2 or more ascites
- Upper gastrointestinal bleed within the past one month.
- Esophagogastroduodenoscopy showing grade 2 or more esophageal varices
- Intestinal paralysis
- Hepatic or extrahepatic malignancy
- Disseminated intravascular coagulation
- Patients unwilling or unable to give consent or participate in all study requirements

After enrolment in the study, the patients were randomly assigned to 2 groups viz. group A (SOC arm) and group B (FMT arm), using random number generation by SPSS version 21.0.

GROUP A

Patients diagnosed with SAH who fulfil the inclusion criteria were enrolled in this group after taking informed consent and received standard care of treatment. It included abstinence of alcohol, nutritional therapy and steroids. These patients were put on diet containing 1.5 g protein and 40 kcal/kg body weight, supplemented with vitamin B-complex including thiamine and zinc. Oral prednisolone at a dose of 40

mg per day was given and response was assessed on 7th day using Lille score. In case of adequate response, steroids were continued till 28 days and then tapered over next 2 weeks. In case of non-responder steroids were discontinued on 7th day. Pentoxifylline was not used. Prophylactic antibiotics were given in case of suspected sepsis, which were later modified according to culture and sensitivity results.

GROUP B

In this group, along with standard care of treatment as described for group A, enrolled patients were given 3 session of FMT. For FMT solution, donor's 50 gm faeces were diluted in 100 ml of normal saline. After proper blending, the slurry was filtered and particulate matter removed. The resultant solution was administered to the patient through nasoduodenal tube within 6 hours of preparation. Patients were kept nil per oral 6 hours before and 2 hours post FMT. FMT was given on day 1, day 4 and day 7 after enrolment in study.

INCLUSION CRITERIA FOR DONOR FOR FMT

- Age >18 years
- Score of 2,3 or 4 on Bristol stool scale
- Stool culture and microscopy tests negative for common detectable enteric pathogens (Salmonella, Shigella, Campylobacter, Vibrio parahemolyticus, Vibrio cholera, Candida albicans, E. coli, Clostridium difficile, Entamoeba histolytica, Giardia lamblia, Cryptosporidium, Ascaris lumbricoides, Trematodes/ tape worms)
- Negative screening for hepatitis A, anti HCV, HBsAg, syphilis and anti-HIV within three months of donation.

EXCLUSION CRITERIA FOR DONOR for FMT

- Tattoos or body piercings within the last 6 months
- High-risk sexual behaviours

- Communicable illnesses or antibiotic treatment within the past 3 months
- Intrinsic gastrointestinal illnesses such as irritable bowel syndrome, inflammatory bowel disease, gastrointestinal malignancies or major gastrointestinal surgical procedures.
- Ongoing immune-modulator therapy for any concurrent illness
- A history of chronic pain syndromes (fibromyalgia, chronic fatigue) or neurologic, neurodevelopmental disorders
- Metabolic syndrome, obesity (BMI of >30), or moderate-to-severe undernutrition (malnutrition)
- A history of malignant illnesses or ongoing oncologic therapy

The enrolled patients underwent physical examination, complete blood counts, Liver Function Tests, Kidney Function Tests, International Normalized Ratio, chest X ray, urine routine examination, serum procalcitonin, CRP, arterial ammonia, viral markers, ultrasound abdomen and other relevant investigations to rule out other aetiologies of jaundice. Blood and appropriate body fluid culture was sent in case of suspected sepsis. Esophagogastroduodenoscopy was done in all patients for variceal assessment.

These patients were followed up to 6 months after enrolment or until death, whichever was early. Relevant investigations were repeated as required and on follow up. Patients were evaluated for overall survival and cause of death till 6 months. They were also evaluated on basis of prognostic indices viz. [Child-Turcotte-Pugh score (CTP), model for end-stage liver disease (MELD), MELD sodium (MELD Na), modified discriminant function (mDF), Glasgow Alcoholic Hepatitis Score (GAHS)] at baseline, at the end of 1st and 3rd months.

DURATION OF STUDY

Study was conducted over a period of 18 month. Eligible patients were enrolled in study for 12 months (1st April, 2019 – 31st March, 2020) and followed up till 6 months.

OUTCOME

PRIMARY OUTCOME

1. Proportion of participants with Overall Survival in both groups at 1 and 3 months.

SECONDARY OUTCOME

1. Improvement in Child Pugh Turcotte (CTP), Model for End Stage Liver Disease (MELD), MELD sodium (MELD Na), Glasgow Alcoholic Hepatitis score (GAHS), Maddrey's Discriminant Function (mDF) in both groups at 1 and 3 months
2. Proportions of participants with overall survival in both groups at 6 months.

STATISTICAL ANALYSIS

The clinical response in the two groups was compared at 1, 3 and 6 months. Wilcoxon's Sign Rank tests was performed within groups at each time point for Child Pugh Turcotte (CTP), Model for End Stage Liver Disease (MELD), MELD sodium (MELD Na), Glasgow Alcoholic Hepatitis score (GAHS), Maddrey's Discriminant Function (mDF) as well as between the groups at each time point using Mann-Whitney U tests. The data was entered into MS Excel version 16 and analysed using INC. SPSS software version 21.0 for statistical analysis.

OBSERVATION AND RESULTS

The present study was conducted in Dayanand Medical College and Hospital, Ludhiana in which 24 patients of severe alcoholic hepatitis were enrolled, out of which 4 patients were lost to follow up and remaining 20 patients were included for analysis. Out of total 20 SAH patients, 10 patients were provided standard of care (SOC) and remaining 10 were imparted fecal microbiota transplantation (FMT) along with SOC.

1. PATIENT PARAMETERS

1.1 AGE

Overall, the patient's age ranged from 30-54 years with mean age 41±6.91 years (table 2).

Table 2: Age distribution of the patients

	N	Mean	SD	Minimum	Maximum
Age	20	41.15	6.91	30	54

The maximum patients in SOC group belonged to 30-40 years age group and to 41-50 years age group in FMT group (figure 3).

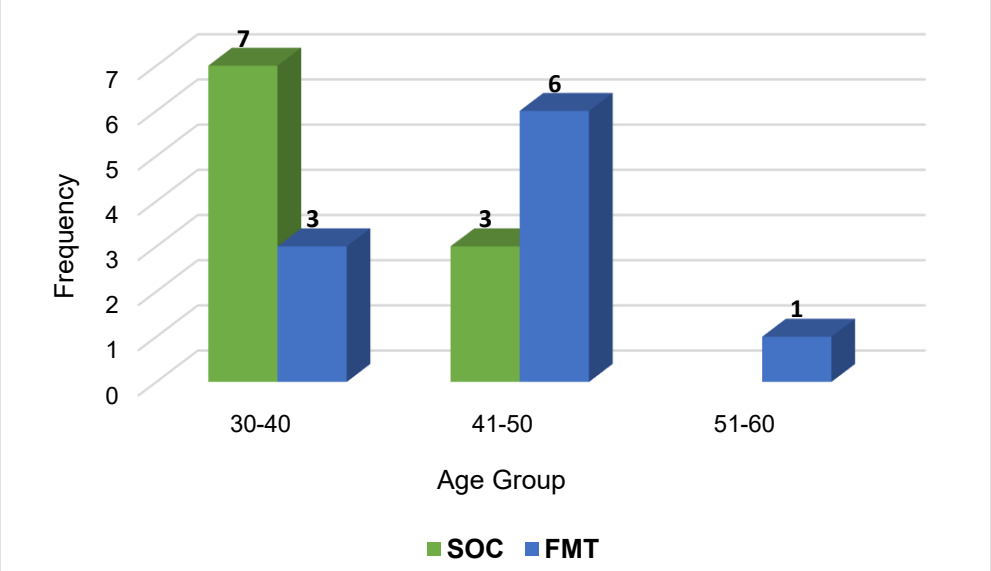

Figure 3: Age distribution of the patients in FMT and SOC groups

Among 10 patients of SOC, the mean age was 39.6±5.64 years, whereas, it was 42.7±7.99 years in FMT group. There was no significant difference in the age of patients of both groups (table 3).

Table 3: Mean age of the patients in FMT and SOC groups

Management	SOC		FMT		t/Z	p-value
Age	Mean	SD	Mean	SD		
	39.60	5.64	42.70	7.99	-1.003	0.329

1.2 DURATION OF JAUNDICE

The mean duration of jaundice among the patients was 16.75±8.68 days with the range of 7-40 days (table 4).

Table 4: Distribution of patients according to duration of jaundice

	N	Mean	SD	Minimum	Maximum
Duration of Jaundice	20	16.75	8.68	7	40

The maximum patients in SOC group had 16-30 days of duration of jaundice, whereas, up to 15 days of jaundice in FMT group.

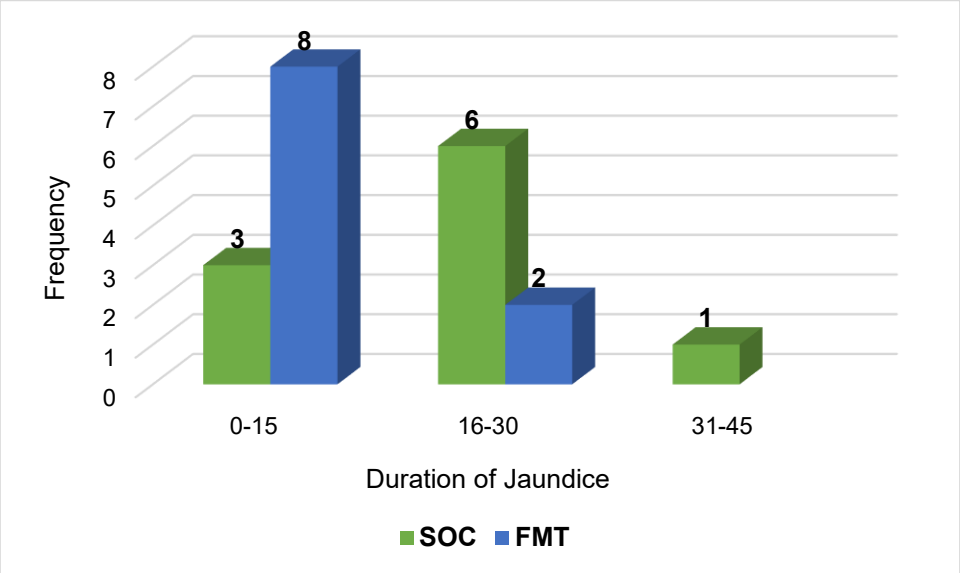

Figure 4: Distribution of patients according to duration of jaundice

There was a significant difference (p- value = 0.026) in the duration of jaundice observed in both groups. Among 10 patients of FMT, the mean duration of jaundice was 21.2±9.89 years, whereas, it was 12.8±4.78 years in SOC group.

Table 5: Mean duration of jaundice in patients of FMT and SOC groups

Management	SOC		FMT		t/Z	p-value
	Mean	SD	Mean	SD		
Duration of jaundice (days)	21.20	9.89	12.80	4.78	2.419	**0.026**

1.3 CO- MORBIDITIES

Out of total, 3 (15%) patients had history of diabetes mellitus, among whom 1 patient belonged to FMT group and 2 patients to SOC group (table 6).

Table 6: Distribution of the patients according to co- morbidities

Clinical History		SOC	FMT	Total	X^2	p-value
Diabetes mellitus	No	9	8	17	0.392	0.531
	Yes	1	2	3		

2. BASELINE PARAMETERS OF THE STUDY PATIENTS

Among the total 20 patients of SAH, mean total bilirubin was 19.79±6.94 mg/dL and mean INR was 2.23±0.55. The AST ranged from 77-208 U/L with mean of 133.4±43.91 U/L, while, mean ALT was 45.4±21.94 U/L. The prognostic scores observed in overall 20 patients included mean CTP of 10.2±1.1, mean MELD of 26.65±3.23, mean MELD Na of 29.25±3.19 and mean mDF was 72.22±28.24. The GAHS score ranged from 6-9 with mean GAHS being 7.75±0.78 (table 7).

Table 7: Baseline parameters in the patients (n=20)

Parameters	Mean	SD	Median	Min- Max
Hb (gm/dL)	11.39	1.20	11.25	9.2-13.6
TLC (10^3/µl)	14885	7886.71	12750	4600-29500
Platelet (10^3/µl)	190.55	109.67	156	60-442
Urea (mg/dL)	15.42	7.65	13.50	4-31
Creat (mg/dL)	0.87	0.27	0.80	0.45-1.40
PT (sec)	23.98	5.89	22.60	18.6-38
INR	2.23	0.55	2.08	1.65-3.51
Na (mEq/L)	131.75	5.35	133	116-138
Total Bil (mg/dL)	19.79	6.94	21.11	7.23-30.70
Direct Bil (mg/dL)	15.59	4.98	16.83	4.73-23.16
AST (U/L)	133.40	43.91	125.0	77-208
ALT (U/L)	45.40	21.94	38.50	17-97
ALP (IU/L)	125.60	39.23	128.50	61-229
Protein (gm/dL)	6.26	0.63	6.25	5.1-7.8
Lactate (mmol/L)	1.57	0.23	1.60	1.2-2.1
CTP	10.20	1.10	10.0	9-12
MELD	26.65	3.23	26.0	20-33
MELD Na	29.25	3.19	30.0	23-36
mDF	72.22	28.24	65.05	34.8-144.8
GAHS	7.75	0.78	8.0	6-9

3. COMPARISON OF PARAMETERS AT BASELINE BETWEEN FMT & SOC

The mean of laboratory parameters was compared between FMT and SOC group at baseline (Table 8).

Table 8: Comparison of Laboratory parameters at Baseline in both groups

Parameters	SOC (n=10)		FMT (n=10)		t/ Z	p value
	Mean	SD	Mean	SD		
Hb (gm/dL)	11.47	1.19	11.32	1.27	0.274	0.79
TLC (10^3/µl)	12450	6710.9	17320	8549.83	-1.417	0.17
Platelet (10^3/µl)	180.8	101.74	200.30	121.79	-0.389	0.70
Urea (mg/dL)	30.5	13.47	31.20	17.70	-0.100	0.92
Creat (mg/dL)	0.85	0.21	0.90	0.33	-0.466	0.65
BUN	15.25	6.73	15.60	8.85	-0.100	0.92
PT	23.93	6.42	24.03	5.67	-0.037	0.97
INR	2.26	0.61	2.21	0.53	0.199	0.84
Na (mEq/L)	132.70	5.06	130.80	5.75	0.785	0.44
K	3.98	0.62	3.50	0.47	1.954	0.07
Cl	96.10	4.91	96.50	4.58	-0.188	0.85
T Bil (mg/dL)	19.85	6.55	19.74	7.67	0.037	0.97
D Bil (mg/dL)	15.68	4.38	15.51	5.76	0.076	0.94
AST (U/L)	138.30	42.96	128.50	46.62	0.489	0.63
ALT (U/L)	36.70	13.89	54.10	25.60	-1.889	0.08
ALP (IU/L)	136.50	39.60	114.70	37.64	1.262	0.22
Protein (gm/dL)	6.58	0.57	5.94	0.55	2.570	**0.02**
Albumin (gm/dL)	2.93	0.40	2.88	0.42	0.270	0.79
Lactate (mmol/L)	1.58	0.30	1.57	0.16	0.091	0.93

It was observed that INR was almost equal in both SOC (mean INR= 2.26±0.61) and FMT (mean INR= 2.21±0.53) group patients. The mean total bilirubin was 19.85±6.55 mg/dL in SOC and 19.74±7.67

mg/dL in FMT group. On comparison of AST and ALT in both groups, mean AST was 138.3±42.96 in SOC and 128.5±46.62 mg/dL in FMT group, whereas, mean ALT was 36.7±13.89 mg/dL and 54.1±25.6 mg/dL in SOC and FMT groups respectively. No significant difference was noted in mean laboratory parameters of both groups at baseline except mean protein level (p- value =0.02), which was more in SOC group (6.58±0.57 gm/dL) than FMT group (5.94±0.55 gm/dL).

Table 9: Comparison of Prognostic scores at Baseline in both groups

Parameters	SOC (n=10)		FMT (n=10)		t/ Z	p-value
	Mean	SD	Mean	SD		
CTP	10.20	1.23	10.20	1.03	0.000	1.000
MELD	26.40	3.27	26.90	3.35	-0.338	0.739
MELD Na	28.90	3.60	29.60	2.88	-0.480	0.637
mDF	71.95	30.88	72.48	27.03	-0.041	0.968
GAHS	7.50	0.53	8.00	0.94	-1.464	0.160

On comparison of prognostic scores in both groups, no significant difference in mean scores of SOC and FMT group patients was observed (Table 9). Mean CTP score was similar in SOC (10.2±1.23) and FMT (10.2±1.03) group. In SOC group, the mean MELD score was 26.4±3.27 and mean MELD Na score 28.9±3.6, which was similar to mean MELD (26.9±3.35) and mean MELD Na score (29.6±2.88) in FMT group. The mean mDF was 71.95±30.88 and 72.48±27.03 in SOC and FMT groups respectively. In SOC and FMT patients, mean GAHS was 7.5±0.53 and 8±0.94 respectively.

4. COMPARISON OF LILLE SCORE BETWEEN FMT & SOC

Overall, the Lille score was ≤0.45 in (55%) patients indicating steroid- responsive at end of 1st week. Among 10 SOC group patients, 6 (60%) patients were steroid responsive (Lille score ≤0.45), whereas, 5 (50%) in FMT group (figure 5).

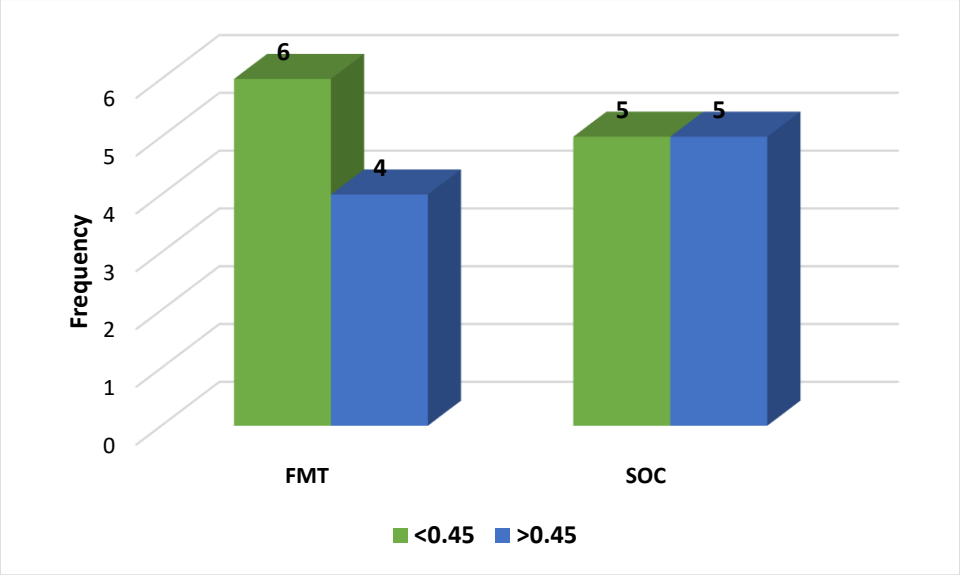

Figure 5: Comparison of Lille score in SOC & FMT groups

There was no significant difference in the mean Lille score between both SOC and FMT groups. The mean Lille score was in SOC and FMT groups respectively (table 10).

Table 10: Comparison of Lille score in SOC & FMT groups

Lille score	t	p-value	Mean Difference	SE Difference	95% CI of the Difference	
					Lower	Upper
1st week	-.747	0.465	-.106	.142	-0.41	0.19

5. COMPARISON OF PARAMETERS BETWEEN FMT & SOC AT 1st MONTH

The various parameters which were compared at the end of 1st month between both groups were survival rate, laboratory parameters and prognostic scores.

5.1 SURVIVAL RATE

In both SOC and FMT group, survival rate was 80% each at the end of 1st month since 2 patients died in both groups each (table 11). There was no significant difference in the survival rate of both groups.

Table 11: Survival rate at end of 1st month in SOC and FMT groups

Survival		**Management**		**Total**	**X^2**	**p-value**
		FMT	SOC			
At 1 month	Died	2 (20%)	2 (20%)	4	7.20	1.0
	Survived	8 (80%)	8 (80%)	16		

5.2 LABORATORY PARAMETERS

A significant difference was observed in mean TLC (p= 0.027), urea (p=0.044), BUN (p= 0.039) at end of 1st month when compared between SOC and FMT group patients.

Mean TLC was higher in FMT (26050±12168.23 $\times 10^3$/µl) than in SOC (14587.50±5040.82 $\times 10^3$/µl) group. In FMT group, both mean urea (62.25±31.57 mg/dL) and BUN (32.29±16.68 mg/dL) was significantly elevated as compared to mean urea (36.25±10.1 mg/dL) and BUN (18.13±5.05 mg/dL) in SOC group. Mean INR was similar in both SOC (2.20±0.86) and FMT (2.66±0.78) patients. On contrary, mean AST observed in SOC (136.63±53.98 U/L) patients was more than FMT (116.50±40.28 U/L) group, whereas, mean ALT was lower in SOC (77.88±54.93 U/L) group than FMT (102.63±90.17 U/L).

Table 12: Comparison of laboratory parameters at end of 1st month

Parameter	SOC (n=8)		FMT (n=8)		t/ Z	p value
	Mean	SD	Mean	SD		
Hb	10.68	0.98	10.68	1.04	0.000	1.000
TLC	14587.5	5040.82	26050	12168.23	-2.462	**0.027**
Platelet	133.75	52.12	173.63	118.77	-0.870	0.399
Urea	36.25	10.10	62.25	31.57	-2.219	**0.044**
Creat.	0.81	0.18	1.09	0.65	-1.192	0.253
BUN	18.13	5.05	32.29	16.68	-2.295	**0.039**
PT	24.01	9.23	29.20	8.59	-1.164	0.264
INR	2.20	0.86	2.66	0.78	-1.097	0.291
Na	132.00	6.19	131.38	8.48	0.168	0.869
K	4.32	0.52	4.36	0.55	-0.135	0.894
Cl	99.00	6.00	100.75	6.90	-0.541	0.597
T Bil.	19.67	12.07	27.39	11.04	-1.336	0.203
D Bil.	14.21	9.21	18.27	5.51	-1.069	0.303
AST	136.63	53.98	116.50	40.28	0.845	0.412
ALT	77.88	54.93	102.63	90.17	-0.663	0.518
ALP	161.25	32.34	147.75	80.45	0.440	0.666
Protein	6.28	1.11	5.94	0.86	0.679	0.508
Albumin	3.11	0.40	3.09	0.43	0.072	0.944
Lactate	1.42	0.22	1.64	0.83	-0.754	0.462

5.3 PROGNOSTIC SCORES

Mean CTP score was 11.5±2.27 in patients who received SOC and 12.5±1.41 in FMT treated patients at end of first month. In SOC group, mean MELD, mean MELD Na and mDF score was 24.75±6.63,

27.25±7.29 and 67.76±47.03 respectively. The patients who received FMT had mean MELD, mean MELD Na and mDF score as 30.75±4.74, 32.13±4.22 and 99.51±38 respectively.

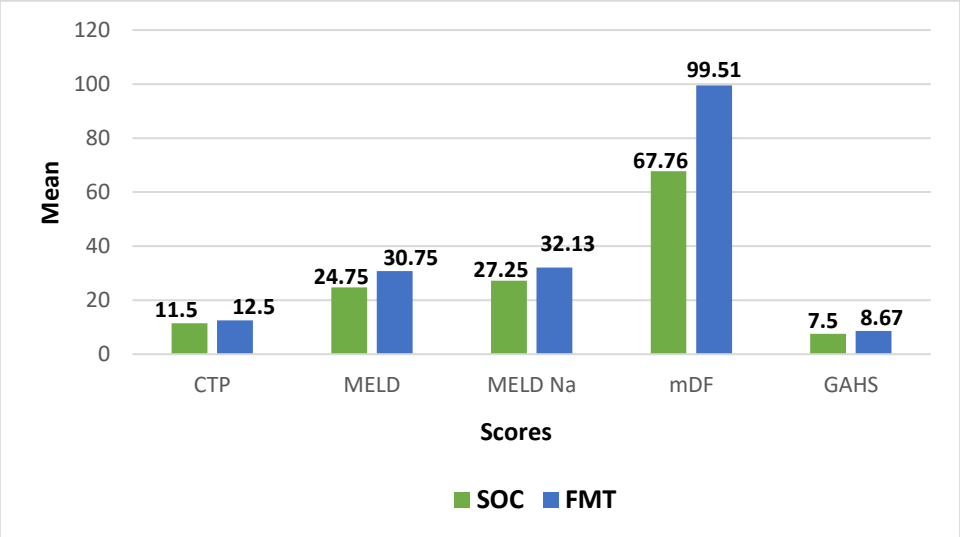

Figure 6: Mean Prognostic scores in both group at 1 month

The difference between mean GAHS score of SOC (7.5±1.2) and FMT (8.67±0.5) group was significant (p= 0.017).

Table 13: Comparison of prognostic scores at end of 1st month

Parameters	SOC (n=8)		FMT (n=8)		t/ Z	p-value
	Mean	SD	Mean	SD		
CTP	11.50	2.27	12.50	1.41	-1.058	0.308
MELD	24.75	6.63	30.75	4.74	-2.082	0.056
MELD Na	27.25	7.29	32.13	4.22	-1.637	0.124
mDF	67.76	47.03	99.51	38.00	-1.485	0.160
GAHS	7.50	1.20	8.67	0.50	-2.684	**0.017**

6. COMPARISON OF PARAMETERS BETWEEN FMT & SOC AT 3rd MONTH

6.1 SURVIVAL STATUS

At the end of 3rd month, 5 patients died in SOC and 7 in FMT group. The survival rate was more in SOC (50%) group as compared to

FMT (30%) group patients (table 14). There was no significant difference in the survival rate of both groups.

Table 14: Comparison of prognostic scores at end of 3rd month

Survival		Management		Total	X^2	P value
		SOC	FMT			
At 3 months	Died	5 (50%)	7 (70%)	12	0.80	0.38
	Survived	5 (50%)	3 (30%)	8		

There was no significant difference in the mean duration of survival (χ^2=0.203; p=0.653) between both group patients at end of 3rd month, where mean period of survival was 8.5±1.3 weeks in SOC and 8.6±1.1 weeks in FMT group.

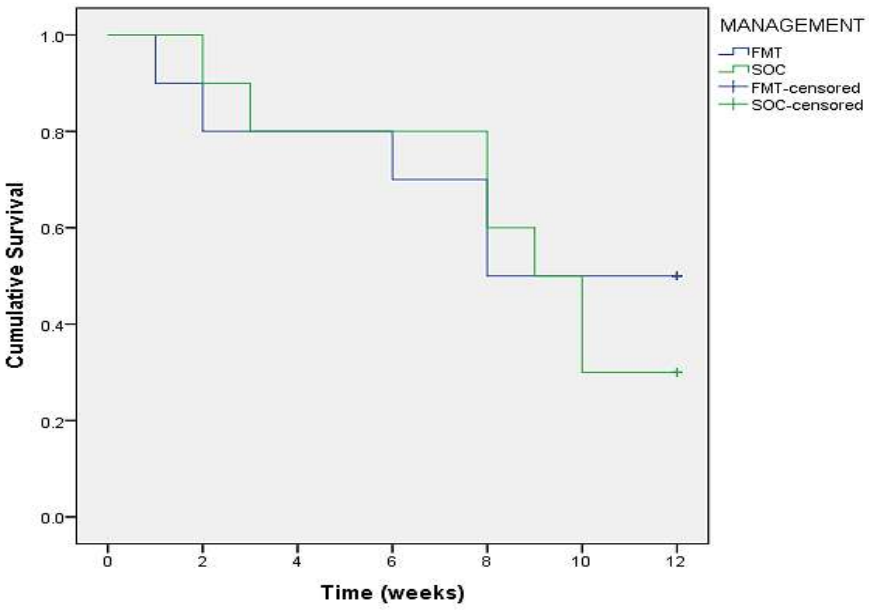

Figure 7: Kaplan Meier graph for survival analysis at 3rd month

6.2 LABORATORY PARAMETERS

At end of 3rd month, significant difference was observed in mean TLC (p= 0.048), urea (p=0.045), BUN (p= 0.026) between SOC and FMT group.

Table 15: Comparison of laboratory parameters at end of 3rd month

Parameter	SOC (n=5)		FMT (n=3)		t/ Z	p-value
	Mean	SD	Mean	SD		
Hb	10.86	1.45	11.17	0.06	-0.355	0.735
TLC	6780	2759.8	11133.3	1450.29	-2.480	**0.048**
Platelet	133.20	22.21	129.33	50.29	0.155	0.882
Urea	31.20	10.89	53.00	13.53	-2.522	**0.045**
Creat	0.84	0.15	0.78	0.18	0.521	0.621
BUN	15.60	5.45	29.75	5.30	-3.121	**0.026**
PT	16.28	2.12	19.00	4.00	-1.291	0.244
INR	1.47	0.21	1.70	0.41	-1.080	0.322
Na	135.80	6.69	129.00	16.46	0.850	0.428
K	4.51	0.79	3.93	0.25	1.191	0.279
Cl	100.40	3.78	98.67	9.45	0.379	0.718
T Bil	6.00	4.10	6.88	1.17	-0.351	0.738
D Bil	4.12	3.29	4.47	0.31	-0.178	0.865
AST	64.40	27.06	76.00	7.55	-0.705	0.507
ALT	39.00	8.75	57.67	28.92	-1.408	0.209
ALP	135.60	43.99	187.67	117.20	-0.931	0.388
Protein	6.54	0.71	6.37	0.50	0.365	0.728
Albumin	3.20	0.42	3.37	0.55	-0.485	0.645
Lactate	1.50	0.33	1.27	0.15	1.140	0.292

Mean TLC was higher in FMT (11133.33±1450.29×10^3/µl) than in SOC (6780±2759.89×10^3/µl) group. In FMT group, both mean urea (53±13.53mg/dL) and BUN (29.75±5.3 mg/dL) was significantly elevated as compared to mean urea (31.2±10.89mg/dL) and BUN (15.6±5.45mg/dL) in SOC group. Mean INR was similar in both SOC (1.47±0.21) and FMT (1.7±0.41) patients. Both mean AST (76±7.55 U/L) and ALT (57.67±28.92 U/L) was higher in FMT group than mean AST (64.4±27.06 U/L) and mean ALT (39±8.75 U/L) in SOC patients.

6.3 PROGNOSTIC SCORES

Various prognostic scores compared between the SOC and FMT patients at the end of 3rd month were CTP, MELD, MELD Na, mDF and GAHS.

Table 16: Comparison of prognostic scores at end of 3rd month

Parameters	**SOC (n=5)**		**FMT (n=3)**		**t/ Z**	**p-value**
	Mean	SD	Mean	SD		
CTP	8.40	0.89	8.67	0.58	-0.455	0.665
MELD	16.20	5.81	19.67	2.08	-0.971	0.369
MELD Na	18.60	6.50	22.33	5.86	-0.812	0.448
mDF	22.54	13.28	36.20	17.61	-1.258	0.255
GAHS	6.00	0.00	6.67	0.58	-2.739	**0.034**

Mean CTP score was similar in both SOC (8.4±0.89) patients and in FMT (8.67±0.58) treated patients at end of 6th month. In SOC group, mean MELD, mean MELD Na and mDF score was 16.2±5.81, 18.6±6.5 and 22.54±13.28 respectively. The patients who received FMT had mean MELD, mean MELD Na and mDF score as 19.67±2.08, 22.33±5.86 and 36.2±17.61 respectively.

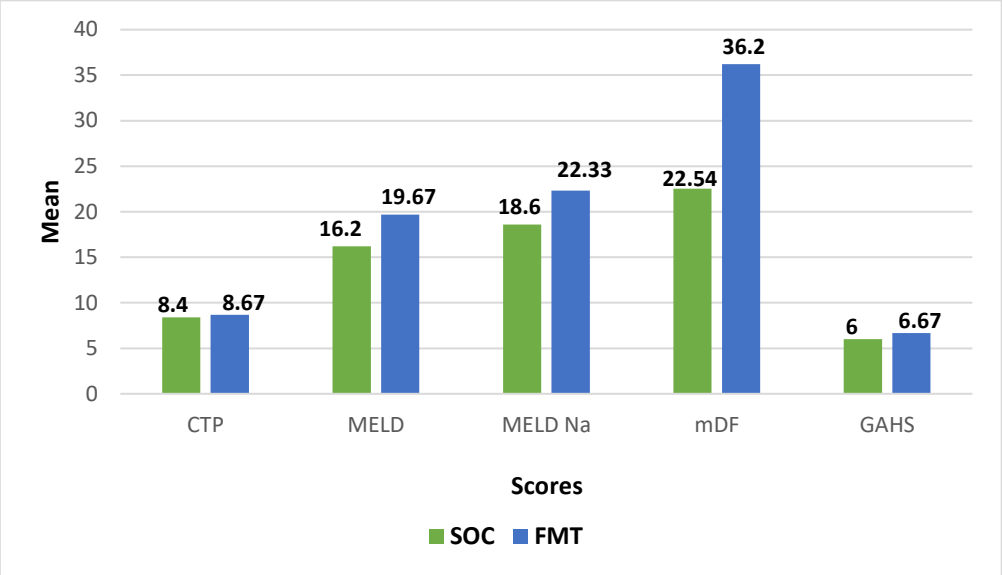

Figure 8: Mean Prognostic scores in SOC and FMT group at 3 months

There was significant difference observed between mean GAHS score in SOC (6) and FMT (6.67) managed patients; p=0.034.

7. SURVIVAL ANALYSIS AT 6 MONTHS

Overall, the mortality rate was lower in SOC (70%) group as compared to FMT (90%) group patients (table 16) at the end of 6th month, since, 7 patients died in SOC and 9 in FMT group. A significant difference was observed in the mortality rate of both groups (p=0.007).

Table 17: Comparison of mortality rate at end of 6th month

Survival		**Management**		**Total**	**X^2**	**p-value**
		SOC	FMT			
At 6 months	Died	7 (70%)	9 (90%)	16	7.20	**0.007**
	Survived	3 (30%)	1 (10%)	4		

There was no significant difference in the mean duration of survival (χ^2=0.475; p=0.491) between both group patients at end of 6th month, though, mean period of survival was higher in SOC (12.1±9.8 weeks) than in FMT group (9.8±1.8 weeks).

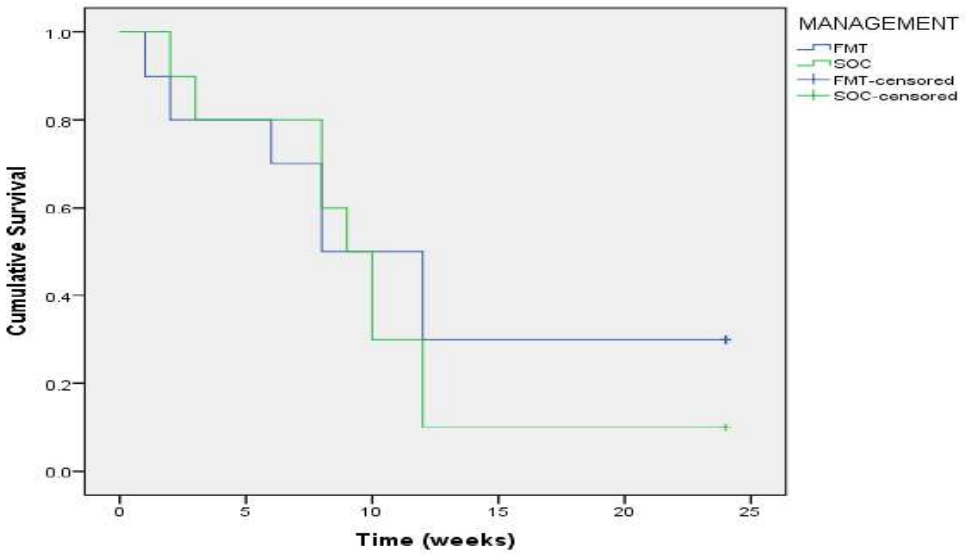

Figure 9: Kaplan Meier graph for survival analysis at 6th month

Number of deaths in both SOC and FMT groups patients during the follow up period is depicted in figure 10. Sepsis with septic shock secondary to bacterial pneumonia was the main cause of death in both groups at end of 1st month.

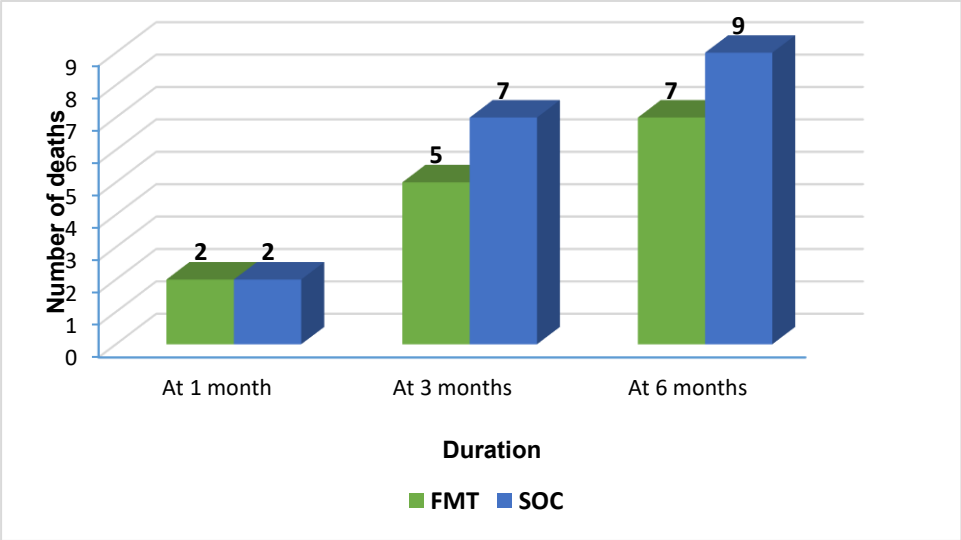

Figure 10: Death of patients at 1, 3, 6 months

Whereas, at end of 3rd month, 4 patients in FMT group and 2 in SOC group died due to bacterial pneumonia and 1 patient each died due to UTI in both groups. In FMT group, 2 patients developed lower limbs cellulitis till 6 months leading to death, while, 2 patients in SOC group died due to bacterial pneumonia.

8. TREND ANALYSIS OF LABORATORY PARAMETERS

The mean bilirubin level initially increased from baseline (19.85 mg/dL) to 1st week (20.58 mg/dL), followed by decrease at 1st (19.67 mg/dL) and 3rd month (6 mg/dL) in SOC group (figure 11).

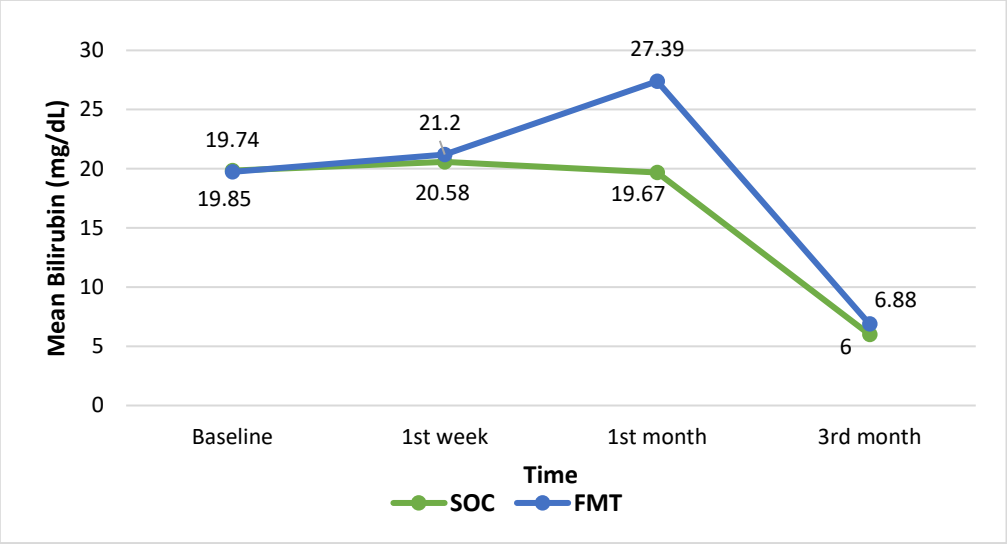

Figure 11: Trend analysis of mean bilirubin in SOC and FMT groups

However, in FMT group, the mean bilirubin gradually raised from baseline (19.74 mg/dL) to 1st month (27.39 mg/dL) and later decreased towards end of 3rd month (6.88 mg/dL).

Table 18: Comparison of mean bilirubin over time

Total Bilirubin	t	p value	Mean Differ	SE Differ	95% CI of the Difference	
					Lower	Upper
Baseline	.037	.971	.117	3.18	-6.58	6.82
1st week	-.17	.865	-.621	3.61	-8.19	6.96
1st month	-1.33	.203	-7.726	5.78	-20.13	4.68
3rd month	-.35	.738	-.875	2.49	-6.97	5.22

No significant difference was seen in mean bilirubin levels between both groups.

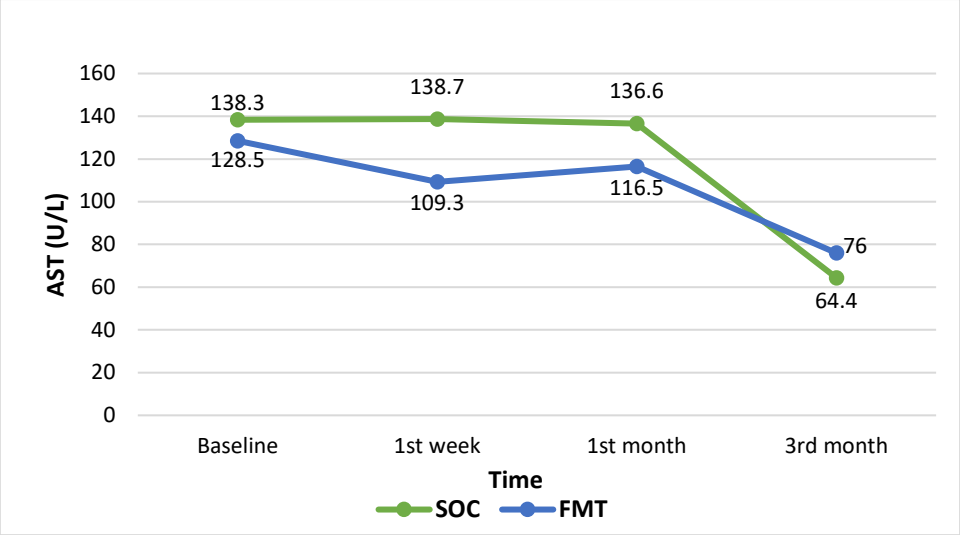

Figure 12: Trend analysis of mean AST in SOC and FMT groups

In SOC group, mean AST was initially stable at baseline (138.3 U/L) till 1st week (138.7 U/L), later decreased towards end of 3rd month (64.4 U/L). However, in FMT group, the mean AST gradually declined from baseline (128.5 U/L) to 1st week (109.3 U/L), further was 76 U/L at end of 3rd month (figure 12).

Table 19: Comparison of mean AST during follow up

AST	t	p-value	Mean Differ	SE Differ	95% CI of the Difference	
					Lower	Upper
Baseline	.489	.631	9.800	20.047	-32.32	51.91
1st week	1.244	.229	29.400	23.627	-20.24	79.04
1st month	.845	.412	20.125	23.812	-30.94	71.19
3rd month	-.705	.507	-11.600	16.447	-51.84	28.65

No significant difference was seen in mean AST levels between SOC and FMT managed patients during follow up period.

Similar trend was noted in mean ALT level from baseline to end of 3rd month in both groups during follow up (figure 13).

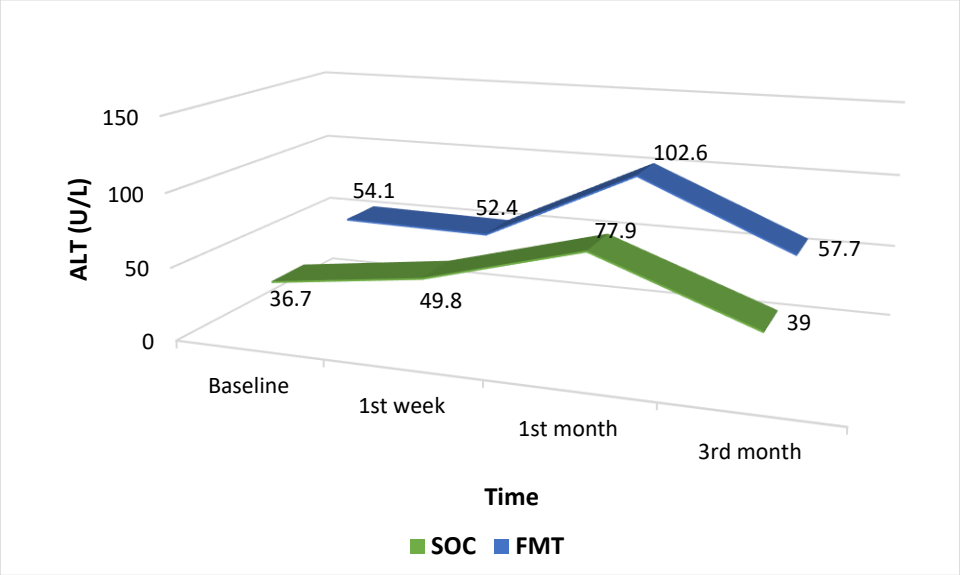

Figure 13: Trend analysis of mean ALT in SOC and FMT groups

Mean ALT was 36.7 U/L in SOC and 54.1 U/L in FMT group, further increased upto 77.9 U/L and 102.6 U/L in SOC and FMT groups respectively. Later it decreased in both SOC (39 U/L) and FMT (57.5 U/L) patients.

Table 20: Comparison of mean ALT during follow up period

ALT	t	p-value	Mean Differ	SE Differ	95% CI of the Difference	
					Lower	Upper
Baseline	-1.889	.075	-17.400	9.209	-36.74	1.95
1st week	-.210	.836	-2.600	12.385	-28.62	23.42
1st month	-.663	.518	-24.750	37.329	-104.81	55.31
3rd month	-1.408	.209	-18.667	13.262	-51.12	13.78

The mean difference between mean ALT levels of SOC and FMT groups was not found to be significant during follow up (table 20).

9. TREND ANALYSIS OF PROGNOSTIC SCORES IN FMT & SOC GROUPS

Mean CTP score was 10.2 in both groups at baseline. It escalated to 11.5 and 12.5 in SOC and FMT groups respectively at end of 1st month, followed by decline in both groups at 3rd month (figure 14).

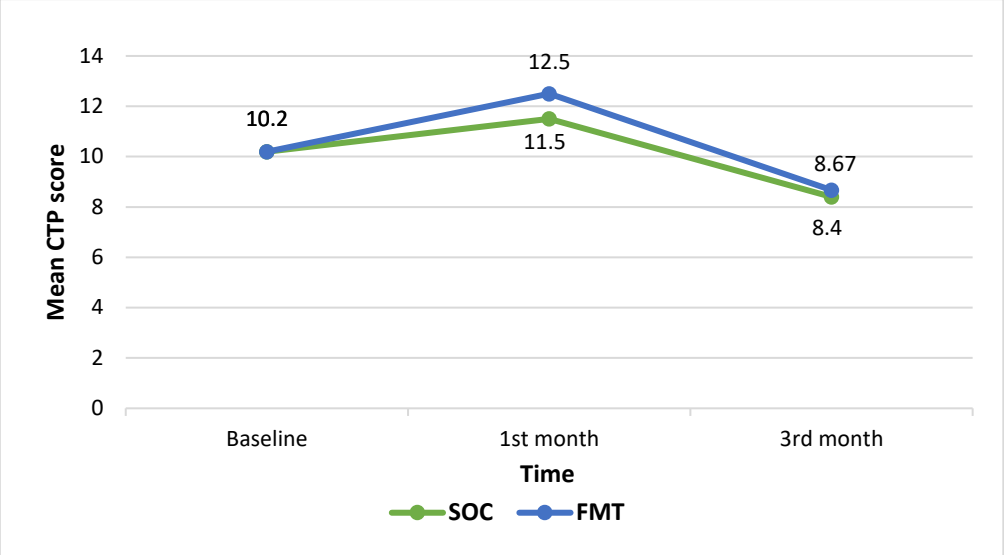

Figure 14: Trend analysis of mean CTP score

Mean CTP score was 8.4 in SOC and 8.67 in FMT group at end of 3rd month.

Table 21: Comparison of mean CTP score in SOC and FMT groups

CTP	t	p-value	Mean Difference	SE Difference	95% CI of the Difference	
					Lower	Upper
Baseline	.000	1.000	.000	.508	-1.060	1.06
1st month	-1.058	.308	-1.000	.945	-3.02	1.02
3rd month	-.455	.665	-.267	.586	-1.70	1.16

On comparison of mean CTP score in both groups over the time period of 3 months, no significant difference was observed between both groups in mean CTP score.

Mean MELD score in SOC group was noted as 26.4±3.273 and 26.9±3.348 in FMT group at baseline. It slightly increased to 24.75±6.628 in SOC patients as compared to greater increase in FMT (30.75±4.743) group (figure 15).

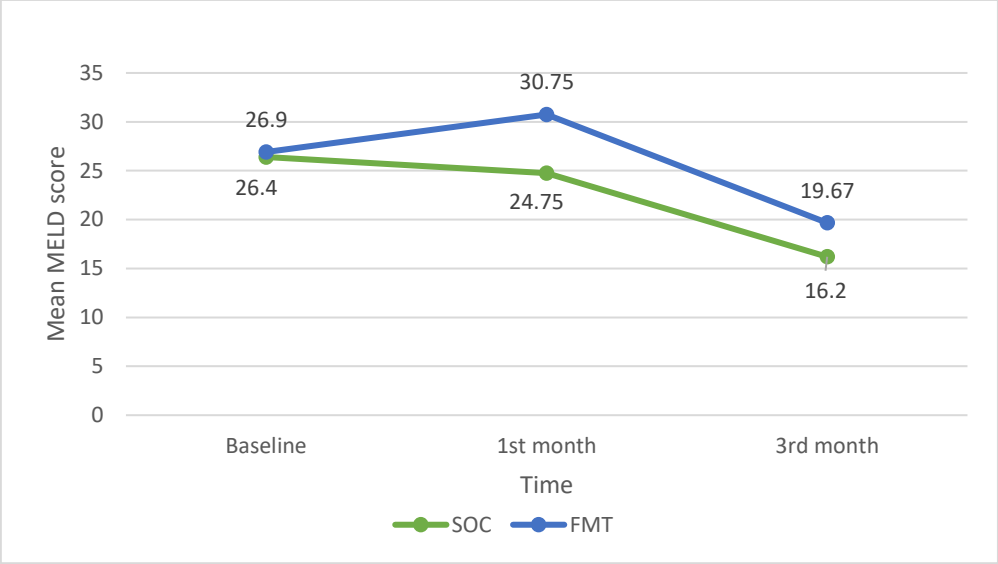

Figure 15: Trend analysis of mean MELD score

At end of 3rd month, mean MELD score was 16.2±5.805 and 19.67±2.082 in SOC and FMT group patients respectively.

Table 22: Comparison of mean MELD score in SOC and FMT groups

MELD	t	p-value	Mean Differ	SE Differ	95% CI of the Difference	
					Lower	Upper
Baseline	-0.338	0.739	-.500	1.481	-3.61	2.61
1st month	-2.082	0.056	-6.000	2.882	-12.18	0.18
3rd month	-0.971	0.369	-3.467	3.571	-12.21	5.27

When mean MELD score was compared in both groups during follow up period of 3 months, no significant difference was observed between both groups in mean MELD score.

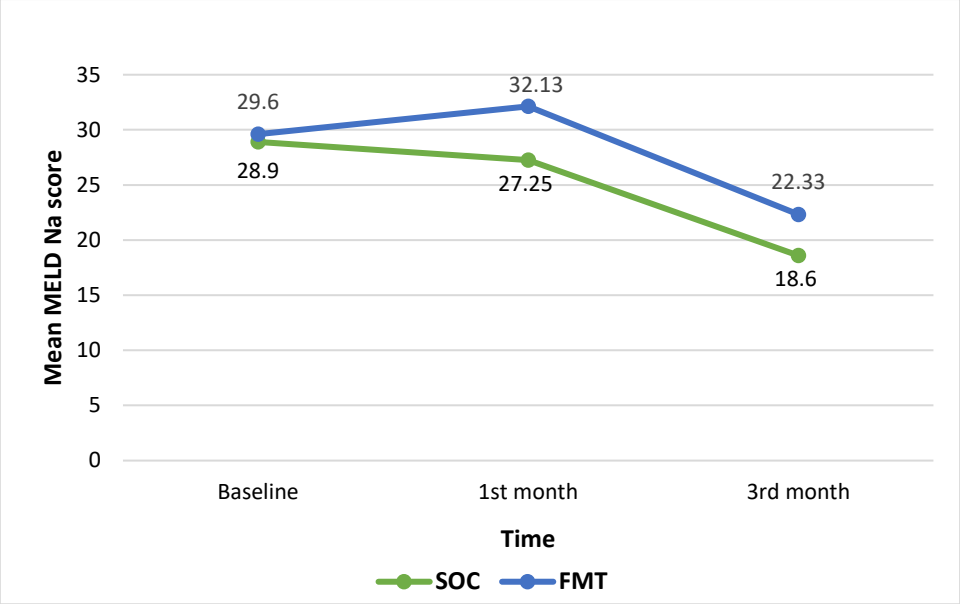

Figure 16: Trend analysis of mean MELD Na score

In SOC group, mean MELD Na score was 28.9±3.604 at baseline, decreased to 27.25±7.285 at 1st month and further declined to 18.6±6.504 at end of 3rd month. Similarly, mean MELD Na score was 29.6±2.875, 32.13±4.224 and 22.33±5.859 at baseline, 1st and 3rd month respectively in FMT group (figure 16).

Table 23: Comparison of mean MELD Na score in SOC and FMT groups

MELD Na	t	p-value	Mean Differ	SE Differ	95% CI of the Difference	
					Lower	Upper
Baseline	-.480	.637	-.700	1.458	-3.76	2.36
1st month	-1.637	.124	-4.875	2.977	-11.26	1.51
3rd month	-.812	.448	-3.733	4.598	-14.98	7.52

On trend analysis of mean MELD Na score in both groups over 3 months duration, no significant difference was documented between both groups (table 23).

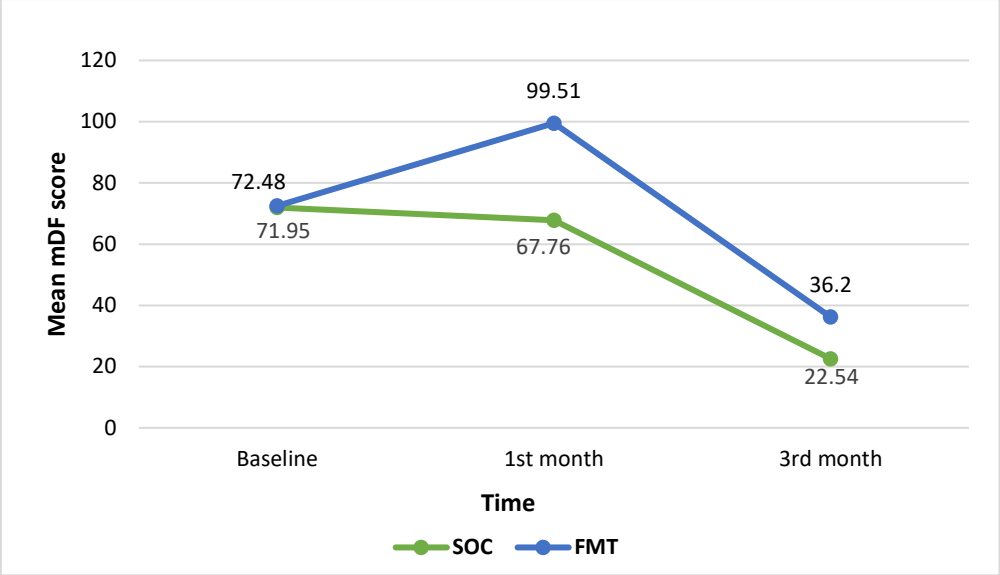

Figure 17: Trend analysis of mean mDF score

Mean mDF score followed a similar trend in both SOC and FMT groups. It was similar at baseline in SOC (71.95±30.88) and FMT (72.48±27.03) patients, followed by increase at 1st month in both groups. At the end of 3rd month, mean mDF score declined to 22.54±13.28 in SOC and 36.2±17.61 in FMT group (figure 17).

Table 24: Comparison of mean mDF score in SOC and FMT groups

mDF	t	p-value	Mean Differ	SE Differ	95% CI of the Difference	
					Lower	Upper
Baseline	-.041	.968	-.534	12.978	-27.80	26.73
1st month	-1.485	.160	-31.750	21.377	-77.60	14.10
3rd month	-1.258	.255	-13.660	10.858	-40.22	12.91

There was no significant difference between mean mDF score of SOC and FMT groups (table 24) during follow up periods.

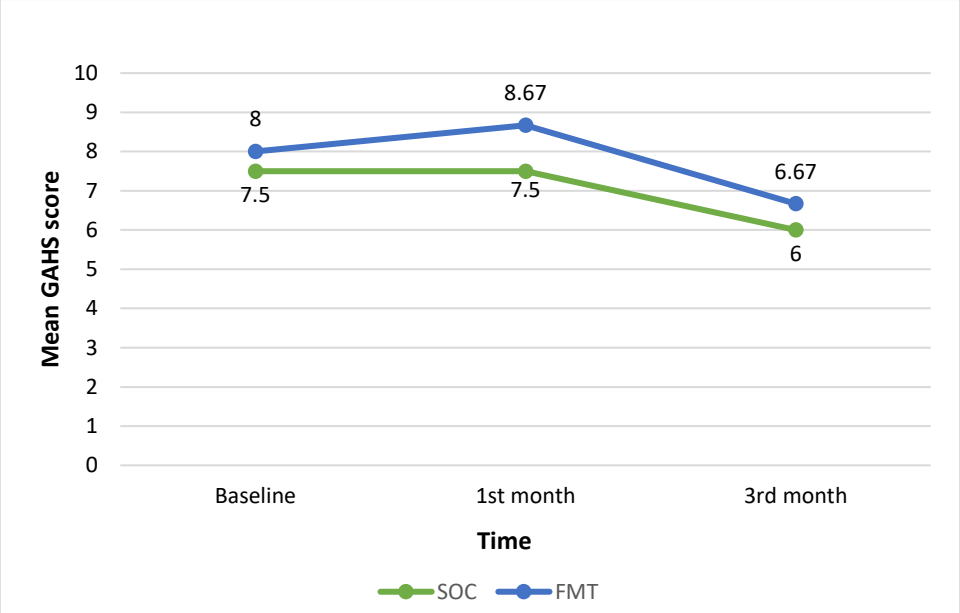

Figure 18: Trend analysis of mean GAHS score

At baseline, mean GAHS score was 7.5±0.53and 8±0.94 in SOC and FMT groups respectively. It increased to 8.67±0.5 in FMT patients, while, was same in SOC patients at 1st month end. Further, mean GAHS score decreased in both SOC (6±0) and FMT (6.67±0.58) groups at end of 3rd month (figure 18).

Table 25: Comparison of mean GAHS score in SOC and FMT groups

GAHS	t	p-value	Mean Differ	SE Differ	95% CI of the Difference	
					Lower	Upper
Baseline	-1.464	0.160	-.500	.342	-1.210	.21
1st month	-2.684	**0.017**	-1.167	.435	-2.09	-.24
3rd month	-2.739	**0.034**	-.667	.243	-1.26	-.07

A significant difference was seen between mean GAHS of both groups at 1st month [t= -2.684; p= 0.017] and 3rd moth end [t= -2.739; p= 0.034]. However, at baseline, there was no significant difference between mean GAHS of both groups (table 25).

DISCUSSION

In the spectrum of ALD, severe alcoholic hepatitis (SAH) remains the most lethal entity due to scarcity of available treatment options. The only definite modality with proved benefits for SAH are steroids, which though, in recent trials were found to improve survival at 1 month only.[8] Even after decades of debate and research, SAH continues to be a treatment enigma, and abstinence remains the only independent predictor of long-term survival. None of the available therapy except liver transplantation has shown promise in improving long term mortality, leaving very few choices in our therapeutic armaments. With better understanding of disease pathogenesis and rapid scientific interest in gut microbiota, fecal microbiota transplantation has been postulated to have therapeutic potential for SAH.[12]

The current study was performed to evaluate the efficacy of incorporating FMT in current standard of care for steroids eligible SAH patients. It was a prospective randomised study, in which group A patients received current standard of care (SOC) that included nutrition therapy and steroids, while patients in group B received FMT in addition of SOC on 1st, 4th and 7th day through nasoduodenal tube. Patients with active sepsis and acute kidney injury were enrolled in study after resolution of sepsis and kidney injury.

Total 20 patients with 30-54 years range, who presented to hospital after 16.75±8.68 mean days from the onset of their jaundice were enrolled and randomized into two groups with 10 patients in each group. Patients in FMT group had significant longer interval between jaundice onset and hospital admission (p=0.026).

Both the groups were identical with respect to laboratory parameters and prognostic scores (CTP, mDF, MELD, MELD- Na, GAHS) at baseline except significantly higher serum total protein (p-value =0.02) in patients of SOC group. The mean mDF was 71.95±30.88 and 72.48±27.03 in SOC and FMT groups respectively at the time of enrolment.

Overall 55% patients in the study (60% in SOC and 50% in FMT group) were found to be steroid responsive based on Lillie score, which were found to be consistent with original Lillie model.(22) FMT incorporation did not improve Lillie score on 7th day in current study. This association is a novel finding of present study since this effect has not been studied in any previous trials.

Mortality in both groups was 20% at 1 month, while at end of 3rd month, 70% mortality was recorded in FMT group and 50% in SOC group. Further, at the end of 6 months, only 1(10%) and 3(30%) patients survived out of 10 patients in FMT and SOC group respectively. STOPAH trial demonstrated 14%, 30% and 57% mortality in prednisolone treated group at 1st month, 3rd month and 1 year respectively [8] while some studies have reported 75% mortality at 6 months in SAH patients.[123] When FMT was used in SAH patients, Phillips et al. observed 25% mortality at end of 1st and 3rd months respectively.[119]

In our current study, FMT has not shown any survival benefits in SAH at 1st, 3rd and 6 months, when administered along with steroids. One previous study has documented survival benefit at 1 year, though in this study the enrolled patients were steroid ineligible and survival was reported to be significantly better in the FMT group (87.5%) compared with historical control group (33.3%).[15] Recently, another study have shown the survival benefit with FMT alone in SAH at 1 and 3 month compared with nutritional therapy, corticosteroids and pentoxifylline group.[119] In this study, they included patients who were steroids responsive and observed 62% mortality in steroid arm compared to 25% in FMT arm at 3 months. In an ongoing randomized controlled trial by Pande et al.,[124] involving the comparison of 112 patients with SAH receiving steroids or FMT, showed an improved 90-day survival rate in the group receiving FMT relative to those given steroids. Above studies were different from our study as FMT was imparted as an additional therapy rather than a separate group in steroid eligible patients.

In current study, we observed higher mortality in FMT group at 3rd and 6th months, where, the mortality was significantly higher at end of 6

months. The main cause of death was sepsis due to bacterial pneumonia; UTI and cellulitis. However, the mean duration of survival was not found to be significantly different between both groups.

Patients of FMT group had significant high level of TLC count and blood urea level at the end of 1st month and this difference also persisted at 3rd month. Prognostic scores (CTP, mDF, MELD, MELD- Na, GAHS) in both groups were similar at 1st and 3rd months except GAHS score which was seen significantly higher in FMT group. This difference may be attributed to change in TLC and BUN value which are component of GAHS. Since bacterial translocation from gut is thought to be the primary mechanism for development of sepsis in SAH patients, the above findings may point towards the potential side effect of FMT. SAH, itself is an immunocompromised state and use of steroids, presence of malnutrition, antibiotics use further enhance the susceptibility for development of sepsis in these patients.

FMT in previous studies has shown to modulate gut microbiota and effect was persistent at 6-12 months.[15] Li et al. quantified the extent of donor microbiota colonization after FMT found extensive coexistence of donor and recipient strains, persisting 3 months after treatment.[125] Modulation of pathogenic bacterial communities to a predominantly non-pathogenic state post-FMT was also demonstrated in SAH patients at 1st and 3rd month by Phillips et al.[119] Since we did not perform qualitative and quantitative microbial community analysis of patients receiving fecal microbiota transplantation in this study, it is difficult to comment upon the association of sepsis and FMT. Moreover, the presence of other confounding factors makes the situation more complex in these patients.

Like every other study, it had its advantages and limitations. This was the first study where efficacy of FMT was evaluated in steroid eligible SAH patients. Patients with advanced liver dysfunction (documented cirrhosis prior to onset of illness, grade 2 or more varices/ ascites at admission) were excluded. The current study is a novel one since both steroids and FMT were given concomitantly. This study also has limitations in form of relatively short sample size, since it included only 10 patients in each group, hence, makes it difficult to extrapolate a

definitive conclusion regarding FMT use in SAH. SAH was diagnosed on the basis of clinical and biochemical parameters and liver biopsy was not performed which would have provided more definite diagnosis. The qualitative and quantitative microbial community analysis of patients receiving FMT was also not performed, which could have provided a better insight of gut dysbiosis.

In the present study, no added benefits of FMT were observed. However, previous conducted clinical trials have shown survival benefits of FMT. It is hard to conjecture whether presence of steroids have blunted the beneficial effect of FMT or FMT has no added advantage in SAH patients. As per present study, the possibility of occurrence of sepsis should be kept in mind while opting for FMT as a treatment modality in SAH patients. Future clinical trials involving larger number of patients with definite diagnosis of SAH and detailed evaluation of gut microbiota will elucidate the existing knowledge and further delineate the role of FMT in SAH.

SUMMARY AND CONCLUSION

The present study was conducted in Dayanand Medical College and Hospital, Ludhiana in which 20 patients of SAH were evaluated in the study, out of which 10 patients were provided standard of care (SOC) and remaining 10 were imparted fecal microbiota transplantation (FMT) along with SOC.

- The mean age of patients was 41±6.91 years ranging from 30-54 years.
- There was a significant difference in the duration of jaundice between both groups where the mean duration of jaundice was 21.2±9.89 years in FMT and 12.8±4.78 years in SOC group (p value=0.026).
- Both the groups were identical with respect to laboratory parameters and prognostic scores (CTP, mDF, MELD, MELD Na, GAHS) at baseline except mean serum protein level, which was significantly more in SOC arm (6.58±0.57 gm/dL) than FMT arm (5.94±0.55 gm/dL) at baseline.
- The mean mDF was 71.95±30.88 and 72.48±27.03 in SOC and FMT groups respectively at baseline.
- 11(55%) of total 20 patients were steroid- responsive (Lille score ≤0.45); among whom 6 patients belonged to SOC and 5 to FMT group.
- FMT group had significant high level of TLC count and blood urea level at the end of 1st month and 3rd month.
- Prognostic scores (CTP, mDF, MELD, MELD- Na, GAHS) in both groups were similar at 1st and 3rd months except GAHS score which was seen significantly higher in FMT group. Mean GAHS score was 7.5±1.2 in SOC and 8.67±0.5 in FMT group at 1 month; and at 3 months it was 6 in SOC and 6.67±0.58 in FMT patients.
- Mortality in both groups was 20% at 1 month, while at end of 3rd month, 70% mortality was recorded in FMT group and 50% in SOC group. Significant difference in mortality was observed at the end of 6 months, where in 1(10%) survived in FMT group in

contrast to 3(30%) in SOC group likely due to high incidence of sepsis.

CONCLUSION

The management of SAH has been explored extensively since decades but no definite treatment has been recommended. Still research is going on for finding new ways to combat this situation effectively.

In present study, the combination of FMT and steroids has not shown any survival benefits in SAH at 1st, 3rd and 6 months when compared to steroids alone. Indeed, we noticed a significant higher leucocyte count at 1st and 3rd month in FMT group that might led to significant higher mortality at 6 months. It is difficult to ascertain at this point that FMT has potential to increase the risk of sepsis because of presence of many coexisting confounding factors in these patients. The future research shall consider this prospective while analysing the role of FMT in SAH.

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Subject: **Approval of Plan of Thesis.**

This is to inform you that the Faculty of Medical Sciences in its meeting held on 29.05.2019 has approved the plan of thesis of **Dr. Ashok Kumar**, a student of your college for **DM Medical Gastroenterology** along with the names of Supervisor/Co-Supervisor as under:-

Topic Plan of Thesis

Efficacy of fecal microbiota transplantation in management of patients with severe alcoholic hepatitis

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The student has to submit the four copies of thesis (along with PDF format) , to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She must directed to attach a photocopy of this letter with the forwarding letter while submitting thesis to the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic,(if any).

S P Singh
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Controller of Examinations

Note :

1. *If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.*
2. *The student may submit the thesis as per the schedule already conveyed i.e for May/June examination 30th Nov of the preceding the year of examination and for Nov/Dec examinations 31st May of the year of examination.*

EFFICACY OF FECAL MICROBIOTA TRANSPLANTATION IN MANAGEMENT OF PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS

THESIS PROPOSAL

FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

DOCTORATE OF MEDICINE

IN

GASTROENTEROLOGY

OF THE

BABA FARID UNIVERSITY OF HEALTH SCIENCES

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APRIL, 2019

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ABSTRACT OF PLAN OF THESIS

Title	**EFFICACY OF FECAL MICROBIOTA TRANSPLANTATION IN MANAGEMENT OF PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS**
For the degree of	D.M. (Gastroenterology)
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Introduction

Severe alcoholic hepatitis (SAH) is the most aggressive form of alcohol related liver disease. Corticosteroids remain the only available therapy, with a short term response seen in about 60% of patients but not associated with long term survival benefit leaving the liver transplant to be only curative option for these patients. As alcohol induced intestinal dysbiosis is central to the development of the severe alcoholic hepatitis, there is intense effort in recent past to explore the therapeutic potential of modulating gut microbiota.

AIM: To study efficacy of fecal microbiota transplantation (FMT) in management of patients with severe alcoholic hepatitis.

MATERIAL AND METHOD: Enrolled patients will receive 3 sessions of FMT through nasojejunal feeding tube (NJFT) along with standard care of treatment which will be compared with patients receiving only standard care of treatment. The outcome will be analyzed at the end of 30, 90,180 days.

CLINICAL SIGNIFICANCE: With the current available therapeutic options, SAH is associated with very high mortality rate. This study, by analyzing the efficacy of FMT in management of patients with SAH; will try to reveal its future therapeutic options.

Keywords: severe alcoholic hepatitis; fecal microbiota transplantation; corticosteroids

Candidate's Sign Co-supervisor's Sign Supervisor's Sign Principal's Sign

CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis titled **"EFFICACY OF FECAL MICROBIOTA TRANSPLANTATION IN MANAGEMENT OF PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS"** exist in the Department of Gastroenterology, Dayanand Medical College and Hospital, Ludhiana and will be provided to the candidate. We will see that the data being included in the thesis are genuine and is collected by the candidate himself/herself under our supervision and guidance.

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5	**TITLE OF THE TOPIC**	EFFICACY OF FECAL MICROBIOTA TRANSPLANTATION IN MANAGEMENT OF PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS

6. BRIEF RESUME OF THE INTENDED WORK

6.1 NEED FOR THE STUDY

Alcohol-related liver disease is a leading cause of morbidity and mortality, responsible for 0.9% of all global deaths and 47.9% of all liver cirrhosis-attributable deaths.[1] Alcoholic liver disease (ALD) includes a broad spectrum of entities from simple steatosis, acute alcoholic hepatitis with or without underlying cirrhosis, to hepatocellular carcinoma as a complication of cirrhosis.

Alcohol related liver disease is associated with significant change in gut microbial composition and it is now generally accepted that the intestinal flora and gut derived toxins play a critical role in the disease pathogenesis.[2-5] The transmissibility of ethanol-induced liver disease has been demonstrated using faecal microbiota transfer in preclinical models. It is the leaky intestinal barrier in alcoholics which permit translocation of viable bacteria and microbial products to liver, where they induce and promote inflammation causing hepatocytes damage and death. Alcoholics and patient with alcoholic hepatitis display higher levels of bacterial products in their blood than the healthy humans. The stage of liver disease correlates closely with the severity of intestinal dysbiosis.[2]

Severe alcoholic hepatitis (SAH) is the most aggressive form of the disease with a 30 day mortality rate of 20-50% [6] and 3 month mortality of ≥30-70%.[7] SAH is characterized clinically by rapid onset of jaundice and liver failure - coagulopathy, ascites, and hepatic encephalopathy. Its laboratory criteria are defined as modified Maddrey discriminant function (mDF) ≥32 or Model for End-Stage Liver Disease (MELD) ≥21 and/or hepatic encephalopathy.[8] It

characterized by dysregulated and uncontrolled systemic inflammatory response followed by weak compensatory anti-inflammatory response that leads to increased susceptibility to infection and multiple organ failure. Systemic inflammation and poor regeneration are hallmark of SAH.

The management of SAH patients currently relies on providing adequate nutrition, corticosteroids and pentoxifyllin. However, recent studies have shown that corticosteroids improve survival in 28 days but not associated with any mortality benefit in long term at 90 days or 1 year.[9] There are substantial number of patients who do not responds to steroids, a number of patients are not candidate of steroid therapy due to obvious contraindication. They are also associated with significant increase risk of infection. Pentoxifyllin has been used traditionally in steroids ineligible group but studies has failed to show any survival benefit with this agent in SAH.[9,10] Liver transplantation is considered the last option in the wake of medical treatment failure or its contraindication, which in resource and organ scarce country like India does not seems feasible easily. So given the high mortality associated with SAH and lack of available treatment options there is urgent need for new thereupetic modality which may improve outcome in these patients.

Recent studies results of the survival benefit of FMT at 1 year in steroids enligible SAH have raised the hope that FMT might be a valuable future tool in this group.[11] This study aims to find the role of FMT in SAH patients who are steroids eligible and its long term outcome.

6.2 REVIEW OF LITERATURE

The rapid scientific interest in gut microbiota has coincided with a global increase in prevalence of liver disease. The gut-liver axis has attracted greater attention in recent years where intense efforts have been made to study gut microbiota in order to understand disease mechanisms and explore the therapeutic potential of modulating it.

FMT which entails the transfer of a microbial community from a healthy donor to a patient has emerged as a promising treatment option for a range of chronic disorders. The term FMT has replaced earlier names such as bacteriotherapy or human probiotic infusion after documentation that infusion of donor fecal material results in prompt and sustained engrafment of donor bacteria.[12]

A study done by Li et al. to quantify the extent of donor microbiota colonization, in which they monitored strain populations in fecal samples from a recent FMT study on metabolic syndrome patients using single-nucleotide variants in metagenomes. In this study they found extensive coexistence of donor and recipient strains, persisting 3 months after treatment.[13]

In alcohol-related liver disease the gut microbiome has been well characterised, showing an increase in Proteobacteria and Fusobacteria and a reduction in Bacteroidetes and Lactobacillus species. Lachnospiraceae and Ruminococcaceae families are decreased whereas Enterobacteriaceae, Alcaligenaceae, and Fusobacteriaceae are significantly increased in cirrhosis.[14] Alcoholic hepatitis related dysbiosis was shown to be associated with an increase in Bifidobacteria, Streptococci and Enterobacteria and a decrease in anti-inflammatory Clostridium leptum or Faecalibacterium

prausnitzii. The density of these protective strains inversely correlated with certain clinical parameters such as bilirubin levels.[15] Studies with FMT show that there is a clear microbiota signature in severe alcoholic hepatitis, a specific 'pathobiont' possibly exists.[11] Studies to identify these microbial species would open the door for new targets and therapies.

The goal of microbiome centered therapies is restoring microbial ecology. This can be achieved by untargeted approaches including changes in diet, supplementation with beneficial bacteria, antibiotics and FMT. Targeted approach uses bacterial and host metabolites as target.

FMT in recent times has emerged as an option for treatment of liver disease. The proposed mechanism of action involves the restoration of non-pathogenic microbes in gut and resultant production of antimicrobial substances like bacteriocins by these microbes. The mucosa-associated invariant T cell is found to be depleted and have shown a defective antibacterial cytokine response in SAH patients. FMT enhances number and function of these cells and imparts beneficial effects.[4] FMT in pilot studies have been shown to restore gut flora, decreases severity and improve survival in a selected group of patients with SAH. Also compared to steroids FMT has less side effects.

In a pilot study of FMT in steroid eligible patients, Philips et al. demonstrated an improvement in 1-year survival rate in FMT-treated patients compared to historical controls (87.5% vs. 33.3%).[11] The FMT was given daily for 7 consecutive days in 8 patients. In patients with severe alcoholic hepatitis, a relatively high abundance of Proteobacteria and low abundance of Actinobacteria was seen compared to controls. A year after FMT, there was a reduction in Proteobacteria and an increase in Actinobacteria. Coexistence of

donor and recipient species was seen 6–12 months post-FMT. The relative abundance of several species was altered, notably including the pathogenic species Klebsiella pneumoniae (10% to <1% at 1 year), and non-pathogenic species, such as Enterococcus villorum (9% to 23% at 6 months), Bifidobacterium longum (6% to 50% at 6 months), and Megasphaera elsdenii (10% to 60% at 1 year).

The baseline deranged metabolic milieu of patients with severe alcoholic hepatitis; the upregulated methane metabolism, fluorobenzoate acid degradation pathway (mediated by Pseudomonas and Escherichia coli groups) and bacterial invasion of the epithelial cells were downregulated 1-year post-FMT. Similarly, bile secretion, carotenoid biosynthesis, and pantothenate biosynthesis pathways, which were downregulated at baseline, improved to near normal levels following FMT. Moreover, the pathways associated with inflammation (arachidonic acid metabolism), cellular toxic and oxidative stress (geraniol and styrene degradation), endogenous toxic alcohol and volatile organic compound burden (caprolactam, toluene, and propanoate metabolism), and aromatic amino acid generation (tryptophan and phenylalanine metabolism) which were highly active at baseline in patients with severe alcoholic hepatitis, were markedly reduced and modified into beneficial pathways – non-aromatic amino acid biosynthesis, nitrogen metabolism, anti-inflammatory pathways (thiamine metabolism, peroxisome, and peroxisome proliferator activated receptor pathway), and cellular regeneration.

The spectacular success of FMT in the treatment of rCDI has encouranged many clinical investigators to consider the same restorative approach in liver

disease which are associated with varying degree of intestinal dysbiosis. However, most patient with SAH are critically ill and require antibiotic therapy, using FMT in these patients may have negative consequences. This is relatively a new approach and we have a limited data on this where recent studies had shown a promising results. This study aims to delineate the role of FMT in SAH patients and may help to understand the future prospective of FMT.

6.3 AIMS AND OBJECTIVES

To study the efficacy of FMT in management of patients with severe alcoholic hepatitis.

7. MATERIALS AND METHODS

STUDY DESIGN

This prospective study will be conducted on the patients of severe alcoholic hepatitis admitted in Department of Gastroenterology, Dayanand Medical College and Hospital, Ludhiana, India.

Patients diagnosed with SAH during study period will be enrolled for the study. Patients will be divided alternatively in two groups (A and B). Patients will be educated about FMT through a Patient Information Sheet.

The study will be conducted in two groups.

GROUP A

Patients diagnosed with SAH who fulfill the inclusion criteria will be enrolled in this group after taking informed consent. Patients will be given standard care of treatment along with FMT. FMT will be done on day 1, day 4 and day 7. FMT will be done through NJFT (Nasojejunal Feeding Tube).

Donor faeces 50 gm will be diluted in 100 ml normal saline. After proper blending, the slurry will be filtered and particulate matter will be removed. The resultant solution will be administered to the patient through NJFT within 6 hours of preparation.

GROUP B

Patients diagnosed with SAH who fulfill the inclusion criteria will be enrolled in this group after taking informed consent and will receive standard care of treatment alone.

7.1 SOURCES OF DATA

Patients diagnosed with SAH during study period who are admitted in the Department of Gastroenterology, DMC & H will be enrolled for the study.

7.2 METHODS OF COLLECTION OF DATA

The enrolled patients will undergo physical examination, complete blood counts, chest X ray, serum procalcitonin, CRP, Liver Function Tests, Kidney Function Tests, International Normalized Ratio, arterial ammonia, viral markers and other relevant investigations to rule out other etiologies of jaundice.

Appropriate prognostic indices [Child-Turcotte-Pugh score (CTP), model for end-stage liver disease (MELD), modified discriminant function (mDF), Glasgow Alcoholic Hepatitis Score (GAHS)] will be calculated at 1 and 3 months.

INCLUSION CRITERIA

- Patients with SAH (mDF ≥32)
- Patients who are eligible for steroids therapy

EXCLUSION CRITERIA

- Upper gastrointestinal bleed within the past one month.
- Active sepsis
- Acute pancreatitis
- Serum creatinine > 1.5 mg/dl
- Ultrasound evidence of grade 2 or more ascites
- Upper G.I. endoscopy showing grade 2 or more esophageal varices
- Intestinal paralysis
- Hepatic or extrahepatic malignancy
- Disseminated intravascular coagulation
- Patients unwilling or unable to give consent or participate in all study requirements

INCLUSION CRITERIA FOR DONOR

- Age >18 years
- Score of 2,3 or 4 on Bristol stool scale
- Stool culture and microscopy tests negative for common detectable enteric pathogens (Salmonella, Shigella, Campylobacter, Vibrio parahemolyticus, Vibrio cholera, Candida albicans, E. coli, Clostridium difficile, Entamoeba histolytica, Giardia lamblia, Cryptosporidium, Ascaris lumbricoides, Trematodes/ tape worms)
- Negative screening for hepatitis A, anti HCV, HBsAg, syphilis and anti HIV within three months of donation.

EXCLUSION CRITERIA FOR DONOR

- Tattoos or body piercings within the last 6 months
- High-risk sexual behaviors
- Communicable illnesses or antibiotic treatment within the past 3 months
- Intrinsic gastrointestinal illnesses such as irritable bowel syndrome, inflammatory bowel disease, gastrointestinal malignancies or major gastrointestinal surgical procedures.
- Ongoing immune-modulator therapy for any concurrent illness
- A history of chronic pain syndromes (fibromyalgia, chronic fatigue) or neurologic, neurodevelopmental disorders
- Metabolic syndrome, obesity (BMI of >30), or moderate-to-severe undernutrition (malnutrition)
- A history of malignant illnesses or ongoing oncologic therapy

DURATION OF STUDY

Study will be conducted over a period of 18 month. Eligible patients will be enrolled in study for 12 months (1st April, 2019 – 31st March, 2020) and followed up till 6 month.

OUTCOME

Primary outcome

1. Proportion of participants with Overall Survival in both groups at 1 and 3 months.

Secondary outcome

1. Improvement in Child Pugh Turcotte (CTP), Model for End Stage Liver Disease (MELD), Glasgow Alcoholic Hepatitis score (GAHS), Maddrey's Discriminant Function (score) severity in both groups at 1 and 3 months
2. Proportions of participants with overall survival in both groups at 6 month.

STATISTICAL ANALYSIS

The clinical response in the two groups will be compared at 1, 3 and 6 months. Wilcoxon's Sign Rank tests will be performed within groups at each time point for Child Pugh Turcotte (CTP), Model for End Stage Liver Disease (MELD), Glasgow Alcoholic Hepatitis score (GAHS), Maddrey's Discriminant Function (score) as well as between the groups at each time point using Mann-Whitney U tests. The data will be entered into MS Excel and analysed using SPSS software version 20.0. for statistical analysis.

7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.

- Yes, the study requires both investigations and interventions.
- Pre-treatment tests include
    - Complete blood counts, Erythrocyte Sedimentation Rate
    - Renal function test
    - Liver function test
    - PT/INR
    - Random blood sugar
    - Anti HCV antibody, HBs Antigen, Anti HIV antibody
    - Ultrasound amdomen, chest X-ray
    - Procalcitonin level
    - Other relevant investigation
- The following intervention will be carried out
    - In group A, faecal microbiota solution prepared from donor faeces will be given via NJFT to the patients after taking an informed consent.

7.4 HAS ETHICAL CLEARANCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?

Attached herewith.

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PERFORMA (GROUP A / B)

Name	Serial no.
Age / sex	Mrd no.
Address	Phone no.
DOA:	DOD:

1. HISTORY

1.1 Presenting complaints

Symptoms	Duration

1.2 past history

h/o upper g.i. bleed ----------

h/o encephalopathy ----------

h/o jaundice ----------

h/o diabetes, hypertension ----------

h/o ischaemic heart disease/TB ----------

Signature of Candidate **Signature of Supervisor**

1.3 Personal history

Addiction	Quantity	Duration
Alcohol		
Smoking		
Opium		
Others		

2. GENERAL PHYSICAL EXAMINATION

Parameters	Results	Parameter	Results
Blood pressure		Pallor Icterus	
Pulse rate		Cyanosis	
Respiratory rate		Clubbing	
Temperature		Lymph nodes	

3. Systemic examination

Per abdomen	
Resp. system	
CVS	
CNS	

4. Clinical diagnosis .....
.....
.....

Signature of Candidate **Signature of Supervisor**

5. Treatment received

Therapy	Duration
Steroids alone	
Steroids plus FMT	

6. Lab parameters:

Parameters	baseline	Day 7	Day 14	1 month	3 month	6 months
Hb (g/dl)						
TLC						
N/L/M/E/B						
Platelets						
PBF for toxic Granules						
PT/INR						
RBS						
Urea						
s. cr						
Na						
K						
Cl						
Bil. Total						
Bil. Conj.						
AST						
ALT						
ALP						
Total prtn.						
Albumin						
GGTP						
Procalcitonin						
Galactomannan						
b-1,3 glycan						
Blood c/s						
Urine c/s						
Ammonia						
HBsAG						
Anti-HCV						
AntiHAV						
Anti-HEV						
HIV						
Crp						

ESR						
CTP Score						
MELD score						
MELD- Na						
mDF						
GAH score						

7. Chest X-ray PA view:

8. USG abdomen:

9. Upper GI endoscopy:

10. Patients follow-up:

11. Outcome:

Signature of Candidate **Signature of Supervisor**

INFORMED PATIENT CONSENT

I.....D/o, W/o, S/o.....

..... am willing to participate in the study "EFFICACY OF FECAL MICROBIOTA TRANSPLANTATION IN MANAGEMENT OF PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS". The procedure with its complications has been explained to me in local vernacular language. I have fully understood that no special tests and extra expenditure in any form, merely for the purpose of thesis is being carried out. I am free to opt out the study any time without assigning any reason.

.....
Signature of the Witness

Name:

.....
Signature of the patient

Signature of Candidate

Signature of Supervisor

मरीज अनुमति फार्म

तिथि _____

मैं _____ पत्नी/पुत्र/पुत्री _____ निवासी _____ इस शोधकार्य "EFFICACY OF FECAL MICROBIOTA TRANSPLANTATION IN MANAGEMENT OF PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS" में भाग लेने के लिए सहमत हूँ। मैं इस शोधकार्य में भाग लेने के लिए अपनी स्वेच्छा से तैयार हूँ। मैं पुष्टि करता/करती हूँ की यह स्वीकृति देने के लिए मुझे कोई आर्थिक प्रलोभन नहीं दिया गया और न ही दबाव डाला गया है। मुझे इस शोधकार्य का उद्देश्य स्पष्ट कर दिया गया है और मुझे यह विश्वास है की यह विज्ञान और मानवता के लाभ के लिए है। मुझे इस शोधकार्य में होने वाले जोखिम के बारे में समझा दिया गया है और मेरी समझ में यह जोखिम बहुत मामुली है।

मैं इस शोधकार्य में भाग लेने के सम्बन्ध में किसी भी समय अपनी अस्वीकृति व्यक्त कर सकता/सकती हूँ और मेरे अस्वीकृति व्यक्त करने का मेरे इलाज पर कोई प्रभाव नहीं पड़ेगा।

_____ _____

गवाह के हस्ताक्षर रोगी के हस्ताक्षर/अंगूठे का निशान

नाम: नाम:

Ashok

_____

विद्यार्थी के हस्ताक्षर

ਮਰੀਜ਼ ਰਜ਼ਾਮੰਦੀ ਫਾਰਮ

ਮਿਤੀ_____

ਮੈ_____ਪਤਨੀ/ਪੁੱਤਰ/ਪੁੱਤਰੀ_____

ਵਾਸੀ _____

"EFFICACY OF FECAL MICROBIOTA TRANSPLANTATION IN MANAGEMENT OF PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS" ਤੇ ਚਲ ਰਹੀ ਖੋਜ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਰਜ਼ਾਮੰਦ ਹਾਂ। ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਮੈ ਆਪਣੀ ਮਰਜ਼ੀ ਨਾਲ ਤਿਆਰ ਹਾਂ। ਮੈ ਇਹ ਦਸਣਾ ਚਾਹੁੰਦਾ ਹਾਂ/ ਚਾਹੁੰਦੀ ਹਾਂ ਕੇ ਇਹ ਰਜ਼ਾਮੰਦੀ ਦੇਣ ਵਾਸਤੇ ਮੈਨੂ ਕਿਸੇ ਵੀ ਤਰਾਂ ਦਾ ਲੋਭ-ਲਾਲਚ ਨਹੀ ਦਿੱਤਾ ਗਿਆ ਅਤੇ ਨਾ ਹੀ ਕੋਈ ਦਬਾ ਪਾਇਆ ਗਿਆ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਦਾ ਮਨੋਰਥ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਭਰੋਸਾ ਦਵਾਇਆ ਗਿਆ ਹੈ ਕੇ ਇਹ ਖੋਜ ਵਿਗਿਆਨ ਅਤੇ ਮਾਨਵਤਾ ਦੀ ਭਲਾਈ ਲਈ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਵਿਚ ਹੋਣ ਵਾਲੇ ਖਤਰੇ ਬਾਰੇ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਯਕੀਨ ਹੈ ਕੇ ਇਹ ਖਤਰਾ ਬਹੁਤ ਮਾਮੂਲੀ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਦਾ ਢੰਗ – ਤਰੀਕਾ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ।

ਮੈ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਬਾਰੇ ਆਪਣੀ ਰਜ਼ਾਮੰਦੀ ਕਿਸੇ ਵੀ ਸਮੇ ਵਾਪਸ ਲੈ ਸਕਦਾ ਹਾਂ/ ਸਕਦੀ ਹਾਂ ਅਤੇ ਮੇਰੀ ਰਜ਼ਾਮੰਦੀ ਵਾਪਸ ਲੈਣ ਦਾ ਮੇਰੇ ਇਲਾਜ ਤੇ ਕੋਈ ਅਸਰ ਨਹੀ ਹੋਵੇਗਾ।

_____
ਗਵਾਹ ਦੇ ਹਸਤਾਖਰ

_____
ਮਰੀਜ਼ ਦੇ ਹਸਤਾਖਰ

ਮਿਤੀ_____

ਮਿਤੀ_____

ਪਤਾ _____

ਪਤਾ _____

_____
ਨਿਰੀਖਕ ਦੇ ਹਸਤਾਖਰ

_____
ਸਿਖਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ

S. No.	NAME	Age	Jaundice (days)	PH	HM	MN	Hb				TLC				Neutrophil				Basophil				Monocyte				Eosinophil			
							Base	1 week	1 month	3 month	Base	1 week	1 month	3 month	Base	1 week	1 month	3 month	Base	1 week	1 month	3 month	Base	1 week	1 month	3 month	Base	1 week	1 month	3 month
1	B	50	15	No	Y	2	10.8	10	8.8	0	19400	18300	14900	0	83	77	83	0	7.1	10.8	5.6	0	8	10.2	9.7	0	0.5	2.4	1.9	0
2	JS	46	10	DM	Y	2	10.8	11.1	10.4	0	24600	32400	41300	0	39	75	94	0	47	17	31	0	10.8	4.2	2.5	0	1.4	2.4	0.1	0
3	AS	30	15	No	Y	2	12.9	11.4	12.5	11.1	23700	28500	38200	11100	78	70	91	88	15	20	4.8	7	2	8	2	3.5	0	2	0.4	0.7
4	MS	54	20	No	Y	2	11.2	12	11.1	0	12200	14600	10900	0	68	79	74	0	20	9	11	0	10	10	12	0	1	1.4	2	0
5	SM	48	14	DM	No	2	9.7	8.2	10.1	0	25200	12900	21500	0	92	75	85	0	2.4	13	7.2	0	5.3	9.9	7	0	0.1	1.6	0.1	0
6	AS	46	7	No	Y	2	13	12.2	10.8	11.2	4800	9200	13600	9700	72	86	79	82	15	10	7.8	10	10	2	9.5	7.8	1.5	1	0.4	0.1
7	GS	32	10	No	No	2	9.2	9.5	11	0	17000	17200	33600	0	65	68	65	0	27	22	32	0	7	6.3	2	0	1	1.1	1.1	0
8	JS	41	7	No	Y	2	12.1	12	0	0	10300	9800	0	0	55	56	0	0	31	28	0	0	10	106	0	0	2.2	3.2	0	0
9	HS	45	10	No	No	2	11.2	11	0	0	6500	8600	0	0	59	59	0	0	26	26	0	0	11	10	0	0	4.2	4.5	0	0
10	NS	35	20	No	Y	2	12.3	11.7	10.7	11.2	29500	31400	34400	12600	83.5	93.8	70	78	8.4	0.1	26	24	7.5	10	0	5.2	0.6	0.1	2	2.8
11	NK	40	30	No	No	1	11.3	12	0	0	5100	10300	0	0	56	70	0	0	29	20	0	0	12	8	0	0	1.4	2	0	0
12	RS	37	10	No	Y	1	10.8	9.3	10.4	0	5300	5400	13700	0	72	87	87	0	13	5	10	0	10	5.3	2	0	4	0.2	1	0
13	SN	40	30	No	Y	1	10.4	9.2	11.3	8.5	11500	9800	12600	5000	72	62.7	82.2	62	16	22.6	12.8	24	10	11.2	3.9	10	2	3.2	1.1	3.5
14	NK	36	40	No	Y	1	12.61	9.6	11.5	10.9	23500	14400	19300	5700	84	84	88	36.8	8	8.5	9	55.4	8	6.6	3.3	5.9	0.4	1	0	1.7
15	SSS	43	20	No	No	1	11.9	11.9	11.4	10.8	13300	17400	11200	5100	72	88	86	68.3	26	10	9	20.8	2	2	3	10.4	0	0	1.8	0.1
16	MK	39	7	DM	Y	1	13.6	11	10.8	12	4600	7200	18200	11600	76	71	90	84	14	19	7	2	10	11	2	1	0.1	0.2	0.8	1
17	SS	48	20	No	No	1	10.1	9.7	11.5	12.1	19700	17200	11600	6500	83	83	66	78	8.2	7	18	18	6.5	7	9.9	2	1.8	2.8	5.4	1
18	NK	48	20	No	No	1	11.8	11.7	9.6	0	12100	4800	7400	0	65	68	78	0	22	21	13	0	9.1	9	8	0	3.5	1	1	0
19	GS	30	15	No	No	1	12.3	12.1	0	0	19800	20800	0	0	72	74	0	0	16	13.7	0	0	10.7	10	0	0	0.5	1.6	0	0
20	RK	35	20	No	No	1	9.9	9.6	8.9	0	9600	11600	22700	0	71.3	80	88	0	16.6	16	8	0	10.31	2	2	0	1	1	1	0

S. No.	NAME	Age	Basophil				Platelet				PT				INR				Urea				BUN				Creatinine		
			Base	1 week	1 month	3 month	Base	1 week	1 month	3 month	Base	1 week	1 month	3 month	Base	1 week	1 month	3 month	Base	1 week	1 month	3 month	Base	1 week	1 month	3 month	Base	1 week	1 month
1	B	50	0.1	0.1	0	0	116	116	100	0	22.6	27.1	37.2	0	2.09	2.51	3.15	0	23	37	59	0	11.5	18.5	29.5	0	1.3	1.2	1.02
2	JS	46	0.7	0.4	0.1	0	351	382	213	0	23	36	46		2.18	3.34	4.32	0	21	19	39	0	10.5	9.5	19.5	0	0.5	1.04	0.9
3	AS	30	0	0	0	0.2	442	392	441	150	27	29.7	28	23	2.5	2.7	2.52	2.11	8	28	32	67	4	14	16	33.5	0.8	0.9	0.69
4	MS	54	0.4	0	0.5	0	100	110	110	0	19	21	22.1		1.78	1.98	2.06	0	25	29	102	0	12.5	14.5	51	0	1.4	1.07	2.51
5	SM	48	0	0.2	0	0	140	91	120	0	22	24	26.5		2.03	2.28	2.43	0	60	45	120	0	30	22.5	60	0	1.12	0.81	1.49
6	AS	46	0.1	1	2.5	0.1	85	82	66	72	18.6	26	27.5	19	1.65	2.41	2.52	1.7	20	17	54	52	10	8.5	27	26	0.6	0.7	0.9
7	GS	32	0.1	0.4	0	0	230	226	199	0	29.1	28.7	27.3		2.67	2.63	2.5	0	29	34	46	0	14.5	17	23	0	0.59	0.85	0.7
8	JS	41	0.1	0.2	0	0	172	250	0	0	22.7	22.1	0	0	2.08	2.03	0	0	41	23	0	0	20.5	21.5	0	0	1.05	0.58	0
9	HS	45	0.5	0.8	0	0	94	90	0	0	37	50	0	0	3.4	4.6	0	0	23	23	0	0	11.5	11.5	0	0	0.6	0.9	0
10	NS	35	0	0	0	0	273	362	140	166	19.3	19.8	19	15	1.72	1.82	1.75	1.3	62	44	46	40	31	22			1.06	0.65	0.5
11	NK	40	0	0	0	0	60	83	0	0	38	28	0	0	3.51	2.6	0	0	42	37	0	0	21	18.5	0	0	1.1	0.9	0
12	RS	37	1	2.5	1	0	100	81	90	0	22.6	29.4	37		2.7	2.67	3.46	0	35	30	40	0	17.5	15	20	0	1.02	0.7	0.6
13	SN	40	0.2	0.3	0	0.3	146	140	126	140	25.9	19.1	14.6	17.3	2.38	1.75	1.34	1.59	14	20	22	26	7	10	11	13	0.8	0.64	0.7
14	NK	36	0	0.5	0.1	0.2	376	299	205	110	18.9	15.5	13.9	14	1.73	1.42	1.28	1.3	46	38	45	22	23	19	22.5	11	1.15	0.72	1.01
15	SSS	43	0	0	0.2	0.4	274	287	216	122	23.9	26.3	19.4	18.4	2.19	2.41	1.72	1.69	15	15	30	22	7.5	7.5	15	11	0.8	0.57	0.73
16	MK	39	0.2	0	0.2	0	166	150	139	126	21	23	28.7	17.7	1.94	2.1	2.63	1.59	18	26	41	43	9	13	20.5	21.5	0.45	0.51	0.9
17	SS	48	0.9	0.3	0.4	1	245	180	121	168	18.8	19.1	15.4	14	1.72	1.75	1.41	1.2	14	14	34	43	7	7	17	21.5	0.65	0.65	0.62
18	NK	48	0.4	0.1	0.2	0	90	80	70	0	31.8	29.8	33.3		2.92	2.73	3.06	0	38	24	26	0	19	12	13	0	0.9	0.7	0.79
19	GS	30	0.8	0.1	0	0	251	205	0	0	19.2	22.2	0	0	1.76	2.04	0	0	41	42	0	0	20.5	21	0	0	0.78	1.2	0
20	RK	35	0.8	1	1	0	100	98	103	0	19.2	27.8	29.8		1.76	2.56	2.73	0	42	49	52	0	21	24.5	26	0	0.8	0.7	1.1

				Sodium				Potassium				Chloride				Total Bilirubin				Direct Bilirubin				AST					
S. No.	NAME	Age	3 month	Base	1 week	1 month	3 month	Base	1 week	1 month	3 month	Base	1 week	1 month	3 month	Base	1 week	1 month	3 month	Base	1 week	1 month	3 month	Base	1 week	1 month	3 month	Base	1 week
1	B	50	0	132	133	130	0	3.03	4.29	3.49	0	100	92	98	0	30.7	33	42	0	23.16	19	18	0	199	231	167	0	97	96
2	JS	46	0	128	131	133	0	4.1	2.99	5.1	0	91	92	104	0	23.5	26.17	37	0	17.6	12.19	25	0	79	59	47	0	41	29
3	AS	30	0.95	133	133	132	110	4.3	3.8	4.2	3.7	96	97	98	88	20	16	14	6.43	16	14.5	10.5	4.4	112	114	146	75	88	86
4	MS	54	0	133	137	139	0	3.51	3.24	4.73	0	98	109	112	0	28.9	27	31.76	0	22.5	13.7	22	0	81	84	121	0	29	31
5	SM	48	0	116	131	112	0	2.8	4.32	4.42	0	92	100	91	0	16	20	38	0	14.1	14.2	25.6	0	185	49	160	0	62	27
6	AS	46	0.8	134	130	138	139	3.39	3.72	3.8	4.2	103	106	108	106	7.23	11.02	16	6	4.73	7.3	12.9	4.2	122	62	94	69	24	30
7	GS	32	0	137	138	136	0	3.23	3.89	4.94	0	101	103	95	0	9.82	14.79	21.52	0	8.6	8.4	16.53	0	88	83	103	0	33	30
8	JS	41	0	130	129	0	0	3.78	5.08	0	0	90	95	0	0	15.2	15.5	0	0	12.7	12.9	0	0	162	161	0	0	37	62
9	HS	45	0	134	133	0	0	3.63	4.03	0	0	100	102	0	0	23.3	27	0	0	16.8	13.9	0	0	171	157	0	0	74	71
10	NS	35	0.6	131	134	131	138	3.26	4.6	4.2	3.9	94	101	100	102	22.7	21.53	18.86	8.2	18.9	13.28	15.62	4.8	86	93	94	84	56	62
11	NK	40	0	126	125	0	0	2.87	2.19	0	0	92	90	0	0	28	30	0	0	21.4	21	0	0	164	164	0	0	67	125
12	RS	37	0	133	133	130	0	3.82	5.1	4.43	0	97	104	99	0	27.6	31	31.07	0	17	19	19.36	0	128	116	113	0	38	40
13	SN	40	0.8	128	135	136	137	3.92	3.9	3.77	4.1	94	97	96	98	13.45	9.66	8.25	5.03	11.66	8.51	6.12	3.05	77	72	73	55	17	18
14	NK	36	0.8	134	140	137	139	3.38	4.1	4.11	3.9	90	106	105	102	20.7	19.13	6.76	1.63	17.5	15.8	5.98	1.21	100	87	112	28	18	36
15	SSS	43	0.82	133	133	133	124	3.79	4.89	4.11	5.85	96	101	99	95	16.46	10.62	23.06	11.65	14.33	9.56	19.1	9.23	172	172	250	103	39	66
16	MK	39	1.1	136	137	132	139	4.28	4.29	3.8	4.1	98	104	102	104	7.68	8.8	22.26	8.6	6.59	8.4	16.7	5.4	134	111	104	68	42	51
17	SS	48	0.7	124	132	137	140	4.9	4.51	5.25	4.6	106	98	104	103	21.52	18.33	5.95	3.1	16.86	9.71	2.98	1.7	188	184	133	68	41	39
18	NK	48	0	137	134	118	0	4.2	3.18	4.22	0	98	95	86	0	15.18	18.93	20.69	0	13.03	12.67	12.67	0	111	101	136	0	38	40
19	GS	30	0	138	136	0	0	4.84	4.2	0	0	100	98	0	0	23.84	28.16	0	0	18.9	18.45	0	0	208	218	0	0	35	45
20	RK	35	0	138	136	133	0	3.8	4.3	4.9	0	90	102	101	0	24.09	31.17	39.29		19.57	22.44	30.8	0	101	162	172	0	32	38

			ALT		ALP				Protein				Albumin				HE			Ascites				CTP			MELD	
S. No.	NAME	Age	1 month	3 month	Base	1 week	1 month	3 month	Base	1 week	1 month	3 month	Base	1 week	1 month	3 month	Base	1 month	3 month	Base	1 month	3 month	Lille score	Base	1 month	3 month	Base	1 month
1	B	50	104	0	97	96	90	0	6.6	6.3	5.9	0	3.15	3.42	3.2	0	0	2	0	0	3	0	0.86	10	13	0	30	34
2	JS	46	18	0	170	151	107	0	5.1	4.5	4.4	0	2.5	2	2.15	0	0	3	0	0	3	0	0.81	10	14	0	27	36
3	AS	30	290	89	74	98	333	323	6.1	6	6.2	6.9	3.3	3.3	3.5	3.9	0	0	0	0	2	0	0.03	10	12	8	29	27
4	MS	54	32	0	131	129	90	0	6.9	6.6	6.5	0	3.2	3.2	3.3	0	0	3	0	1	3	0	0.65	9	13	0	29	36
5	SM	48	68	0	145	101	136	0	6.3	6.6	6	0	2.89	4.02	3.19	0	0	2	0	1	3	0	0.63	10	14	0	26	34
6	AS	46	68	32	142	132	125	120	5.5	4.5	5.6	5.9	2.6	2.26	2.85	2.8	0	0	0	0	0	2	0.42	9	10	9	20	27
7	GS	32	63	0	61	58	119	0	5.7	6.3	7.4	0	2.1	2.75	3.09	0	0	2	0	1	2	0	0.43	12	13	0	26	28
8	JS	41	0	0	135	122	0	0	6	6	0	0	3.2	2.8	0	0	0		0	1		0	0.15	10	0	0	25	0
9	HS	45	0	0	66	75	0	0	5.6	5.6	0	0	2.57	2.14	0	0	0		0	1		0	0.81	12	0	0	32	0
10	NS	35	178	52	126	129	182	120	5.6	5.4	5.5	6.3	3.3	3.31	3.45	3.4	0	0	0	1	2	1	0.1	10	11	9	25	24
11	NK	40	0	0	114	105	0	0	6.2	6	0	0	2.8	3.4	0	0	0		0	1		0	0.69	11	0	0	33	0
12	RS	37	70	0	169	129	215	0	6.8	6.5	5.9	0	2.7	2.8	3.15	0	0	1	0	1	2	0	0.7	12	11	0	30	33
13	SN	40	32	27	137	132	130	96	6.5	6.4	6.9	7.1	2.9	2.9	3.4	3.5	0	0	0	1	2	2	0.06	11	11	9	26	18
14	NK	36	61	40	138	143	163	130	5.8	4.8	5.2	6.1	2.87	2.49	2.76	2.9	0	0	0	1	1	2	0.16	10	10	8	25	17
15	SSS	43	202	49	115	87	130	211	6.5	6.2	5.5	5.9	3.42	3.1	2.69	2.9	0	2	0	0	1	1	0.03	9	13	9	25	24
16	MK	39	42	34	149	106	160	120	6.8	5.5	5.8	6.1	3.6	2.8	2.8	2.9	0	2	0	0	1	1	0.04	9	13	9	22	29
17	SS	48	101	45	119	129	179	121	6.7	6.6	8.7	7.5	3.1	2.81	3.7	3.8	0	0	0	0	0	1	0.23	9	7	7	24	17
18	NK	48	75	0	101	96	124	0	6.8	7.2	6.4	0	2.74	3.59	3.55	0	0	2	0	1	2	0	0.66	12	13	0	29	30
19	GS	30	0	0	94	126	0	0	7.8	7.3	0	0	3	3.1	0	0	1	2	0	1	1	0	0.38	9	0	0	25	0
20	RK	35	40	0	229	210	189	0	5.9	6	5.8	0	2.17	2.63	2.8	0	0	2	0	0	3	0	0.88	10	14	0	25	30

				MELD sodium			mDF			GAHS			Survival (weeks)					
S. No.	NAME	Age	3 month	Base	1 month	3 month	Base	1 month	3 month	Base	1 month	3 month	surv 1	time 1	surv 3	time 3	surv 6	time 6
1	B	50	0	32	35	0	76.7	155.2	0	9	9	0	1	4	0	10	0	10
2	JS	46	0	31	37	0	71.3	156.6	0	8	9	0	1	4	0	8	0	8
3	AS	30	22	30	29	29	86.2	84.8	54.2	9	9	7	1	4	1	12	0	12
4	MS	54	0	31	36	0	60.2	75.7	0	8	8	0	1	4	0	8	0	10
5	SM	48	0	31	36	0	59.24	101.9	0	8	9	0	1	4	0	8	0	8
6	AS	46	19	23	28	20	34.8	84.5	35.4	6	8	7	1	4	1	12	0	12
7	GS	32	0	27	29	0	85.7	89.1	0	9	9	0	1	4	0	9	0	9
8	JS	41	0	29	0	0	61.7	0	0	7	9	0	0	4	0	4	0	2
9	HS	45	0	33	0	0	135.5	0	0	8	0	0	0	3	0	3	0	3
10	NS	35	18	29	27	18	53.5	48.3	19	8	8	6	1	4	1	12	1	24
11	NK	40	0	36	0	0	144.8	0	0	8	0	0	0	3	0	3	0	1
12	RS	37	0	32	35	0	73.6	108.3	0	7	8	0	1	4	0	11	0	6
13	SN	40	17	30	20	19	74.6	17.4	26.7	8	6	6	1	4	1	12	1	24
14	NK	36	8	28	18	12	49.7	12.7	8	8	7	6	1	4	1	12	1	24
15	SSS	43	22	28	27	28	68.4	54.3	38.3	7	7	6	1	4	1	12	0	12
16	MK	39	21	23	31	21	46.3	96.3	30.2	7	9	6	1	4	1	12	0	12
17	SS	48	13	30	19	13	50	18.8	9.5	7	6	6	1	4	1	12	1	24
18	NK	48	0	30	34	0	103.5	115.9	0	8	8	0	1	4	0	10	0	10
19	GS	30	0	26	0	0	54.2	0	0	8	0	0	0	4	0	4	0	4
20	RK	35	0	26	34	0	54.4	118.4	0	7	9	0	1	4	0	8	0	8